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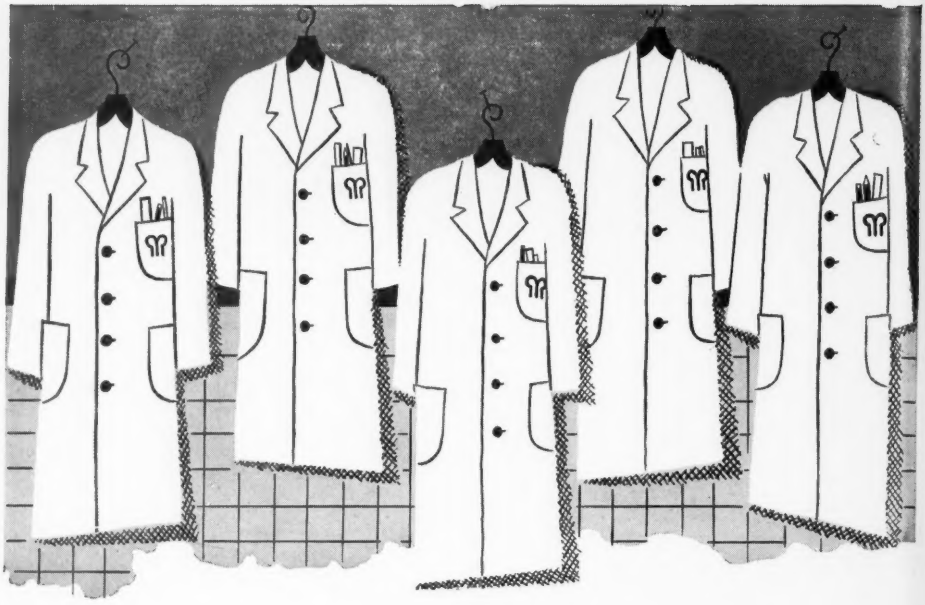

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DIETARY FACTORS IN THE PATHOGENESIS OF SIMPLE GOITER*†

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FOR centuries the cervical swelling known as "goiter" or "bronchocele" has remained a mystery that has intrigued the curious mind of both layman and physician. Although for over a century now it has been generally recognized that the underlying pathology of this condition is an enlargement of a peculiar paratracheal mass of tissue known as the "thyroid gland," little advancement has been made toward a comprehension of the etiology of the disease since the middle of the last century, when Chatin published his classic experiments demonstrating an inverse relationship between the incidence of goiter and the concentration of iodine.

Thanks to the work of such twentieth century pioneers as Marine, McClendon, von Fellenberg, Hercus, and others, the iodine-deficiency theory is now generally accepted, and the governments of some countries where goiter is common have passed, or are now attempting to have passed, legislation which makes the fortification of table salt with potassium iodide mandatory. Such action is highly commendable.

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Although this theory has been demonstrated to be correct by innumerable investigators in innumerable experiments, and the rising use of iodized salt has been accompanied by a concomitant decrease in the incidence of goiter, the undeniable fact remains that not all nontoxic thyroid enlargement can be explained by postulating iodine-lack. It is not at all uncommon to find living in the vicinity of Boston, within a few miles of the ocean, many goitrous individuals who have been in this locality all their lives and who are accustomed to consuming sea food at least once a week. Surely the iodine ingestion in such cases must be well above the 100–200 micrograms estimated to be the daily requirement for man.

With the discovery that certain chemical compounds, the antithyroid drugs, would inhibit the formation of thyroxine, an entirely novel conception of human goitrogenesis was made possible. If such compounds were in some unexplained manner made available to the human organism during his daily routine, the pathogenesis of some heretofore inexplicable cases of thyroid enlargement might now be understood. All that was necessary was the demonstration of a naturally occurring goitrogen in substances that would ensure its access to the thyroid gland. Of course, the most obvious source of such a goitrogen would be in the food or water, and water could be excluded in most modern communities, since municipal water supplies serve the entire population.

The idea that certain foods may cause thyroid enlargement is not new. Many primitive peoples believe that this is the cause of their "swelled necks." No really good experimental evidence in support of this hypothesis was forthcoming, however, until the work of Chesney, Clawson, and Webster in 1928 (1, 2, 3), who discovered that rabbits being maintained in their laboratory were developing large goiters although their necks were perfectly normal upon purchase. After tracing down other possible factors, these investigators decided that the daily cabbage ration was responsible (4).

Marine and his collaborators in New York immediately confirmed and extended the original observations (5, 6), finding that several other Brassicae (the genus to which cabbage belongs) would produce thyroid hypertrophy when fed to rabbits, among them cauliflower and Brussels sprouts. Scientific workers from all over the world added further confirmation to this discovery and several new vegetables were added to the list. A few reports, of course, were made of failure to reduplicate Chesney's results.

No really new contribution was made to the field, however, until 1936, when Hercus and Purves (7) in New Zealand discovered that the seeds of certain of the Brassicae, notably rape seed, mustard seed, and cabbage seed, were effective goitrogens when fed to rats. They also reported an epidemic of goiter occurring among sheep which were fed a diet of turnip

during the winter months. Similar thyroid enlargement was induced in rabbits fed on turnip. Surprisingly enough, however, the New Zealanders were unable to detect any appreciable activity in the native cabbage.

A few years later these same investigators made another important contribution when they discovered that the Brassica seed-goiters would not develop in the absence of an intact pituitary and that the administration of physiologic amounts of thyroid or thyroxine would also inhibit their development. The administration of iodine, however, although lessening the thyroid response to Brassica seed feeding by about one third, would not prevent the goitrogenesis. The mechanism of action therefore was concluded to be an inhibition of thyroxine synthesis (8, 9, 10, 11, 12). At the same time identical conclusions were reached concerning the mechanism of action of the antithyroid drugs (13, 14).

One in particular among many unanswered questions remained to stimulate those interested in the field. What was the nature of the active compound contained in these foods? Twenty years ago Marine had made investigations into this problem and had found that the active principle of cabbage was ether soluble, insoluble in water, volatile, and that the activity of the food could be increased by steaming or boiling. Since the mustard oils are the most characteristic compounds of this family and since they are both ether soluble and volatile, it was only natural that he should have first turned to these isothiocyanates. However, several different mustard oils had no effect when tested in rabbits.

Cyanides were also reported to be common constituents of the cabbage family, so these compounds were next tried. All cyanides tested were found to produce thyroid hypertrophy in the rabbits, but particularly methyl cyanide (acetonitrile), the simplest and least toxic. Marine (15) then postulated that the active material in cabbage was some sort of cyanide and that it produced thyroid enlargement by interfering with oxidation mechanisms in the tissues, thereby in some manner increasing the need for thyroid hormone. Several investigators since then, however, have been unable to confirm the goitrogenicity of cyanide, and at the present time the general consensus is that it is by no means proved that *any* cyanide can be incriminated as a naturally occurring goitrogen.

About this time, another great step forward was taken when Richter and Clisby (1941) in Baltimore reported that a substance they were testing as a taste discriminator, phenylthiourea, would produce thyroid enlargement in their rats (16). The following year Kennedy of the New Zealand group published a short report (17) suggesting that allylthiourea might be the active principle of cabbage and showing that it, too, was goitrogenic in rats. The reasoning behind Kennedy's suggestion was never made quite clear, but probably he put together the facts that allyl mustard oil was common

among the Brassicae, that Richter and Clisby had found phenylthiourea goitrogenic, and that mustard oils are easily converted to thioureas upon the addition of ammonia. Up to 1948, however, no one had been able to isolate from any of these goitrogenic foods a compound which would produce goiter when fed or injected into animals.

In the meantime a series of investigations (18, 19) into the chemical nature of the antithyroid compounds had been undertaken, and it had been found that there were two chief types, those containing a thiocarbonamide grouping, —NH—CS—X—, (X being nitrogen, sulfur, or oxygen) and derivatives of aniline such as sulfaguanidine. The thiocarbonamide type, however, seemed to be the only one effective in man. Reports of goiter occurring in patients treated with thiocyanate, beginning with that of Barker (20) in 1936, led to the discovery that this ion was also a goitrogen. Subsequent work showed, however, that it acted in a much different manner from the compounds discussed above. Thiocyanate apparently inhibited the concentration of iodide ion by the thyroid and had nothing to do with what happened to the iodide once it entered the thyroid cells. Thyroid enlargement caused by this drug could be completely prevented by the administration of iodine, contrary to the very incomplete inhibition exerted by iodine on the action of the other antithyroid drugs. The possibilities were great, then, that the active principle in cabbage and related foods might prove to be one of these three types of compounds. Since the New Zealand workers had shown that the Brassica seed-goiters were only partially inhibited by excess iodine (11), it seemed rather dubious that thiocyanate would eventually prove to be the responsible agent.

It has always been difficult to transfer the results obtained by animal experiment to man. A compound which would be highly goitrogenic for one species might prove to be completely inactive in another. Such is the case with sulfaguanidine or sulfanilamide, which are active in the rat but have never been shown to cause goiter in man in spite of the frequency with which they have been prescribed. Very recently, however, a new method for assaying the antithyroid effect of different compounds in man by the use of radioactive iodine was described (21). It was decided that it might be interesting to apply this technique to testing for the antithyroid activity of various foods in man, since the assayed potency of the drugs agreed well with their relative therapeutic effectiveness.

METHODS AND RESULTS

One hundred microcuries of tracer I^{131} were given to fasting subjects in the morning and the accumulation of radioactivity over the region of the thyroid determined at short intervals by means of an externally placed Geiger-Müller counter. In untreated subjects who are given a tracer dose

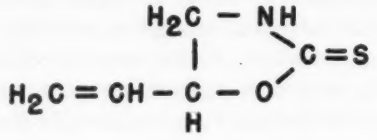
in this manner, the accumulation of radioiodine is a steady and regular process. If an antithyroid compound is given after enough determinations have been made to define the course of iodine collection, the process is slowed or stopped. The degree of inhibition can then be estimated by observing its extent and duration. Foods were tested in this same manner; as soon as the rate of uptake was determined, the subjects were given the food to be tested and instructed to eat to satiety in as short a time as possible. Sixty-one foods were tested in one hundred different subjects by this method, and although several were found to possess some antithyroid activity, rutabaga, or yellow turnip, exhibited by far the strongest and most consistent effect (22).

Since the rutabaga possessed such a high degree of activity, it was decided that this vegetable would furnish suitable material from which to attempt the isolation of a naturally occurring antithyroid substance. First, however, a method had to be devised for assaying the various fractions which would be obtained from the turnip. Repeating the above-mentioned procedure in man with each fraction would be both unnecessarily time consuming and undesirable because of the possibility of administering toxic substances. Consequently, a modification of the method of McGinty *et al.* (23) was chosen. It was found that if the material to be tested were administered to rats by stomach tube or by subcutaneous injection one hour before a tracer dose of I^{131} and the animals killed four hours later, the degree of activity of the fractions could be determined by the depression of I^{131} uptake by the thyroids of the treated animals as compared to the controls.

Progress after this procedure had been worked out was quite rapid. It was found that the activity could be removed from a watery extract of finely ground rutabaga by extraction with ether and that when this ether solution was evaporated, the activity would be taken up in the water-soluble portion of the brownish tar that remained. By serial extraction in this manner, a purified yellow oil was finally obtained which had a characteristic, unpleasant odor. This substance reacted with Grote's reagent in a manner common to the thiocarbonamide compounds and exhibited a peak in the ultraviolet absorption spectrum at 240 mμ which was also consistent with the properties of this type of antithyroid agent.

Further purification of this yellow oil was difficult, but finally, after the material had been distilled under vacuum, it was induced to crystallize. Colorless, odorless, bitter-tasting crystals were obtained which melted at 50°C., were optically levorotatory, $[\alpha]_D^{31} -71°$ (methanol), and were weakly acidic (pK_a10.5). Elemental analysis and molecular weight determinations indicated that the correct empirical formula was C_5H_7ONS. A search through the literature yielded no compound of similar empirical formula and properties.

Largely from physical evidence, it was proved that the antithyroid compound which had been isolated was *l*-5-vinyl-2-thiooxazolidone (Fig. 1)



L-5-VINYL-2-THIOOXAZOLIDONE

FIG. 1. The antithyroid compound isolated from members of the cabbage family.

and contained a thiocarbonamide grouping as had been suspected (24, 25). This substance has now been synthesized in racemic and optically active forms (26) and the levorotatory isomer is identical with the natural product.

The racemic and optically active thiooxazolidones possess equal anti-

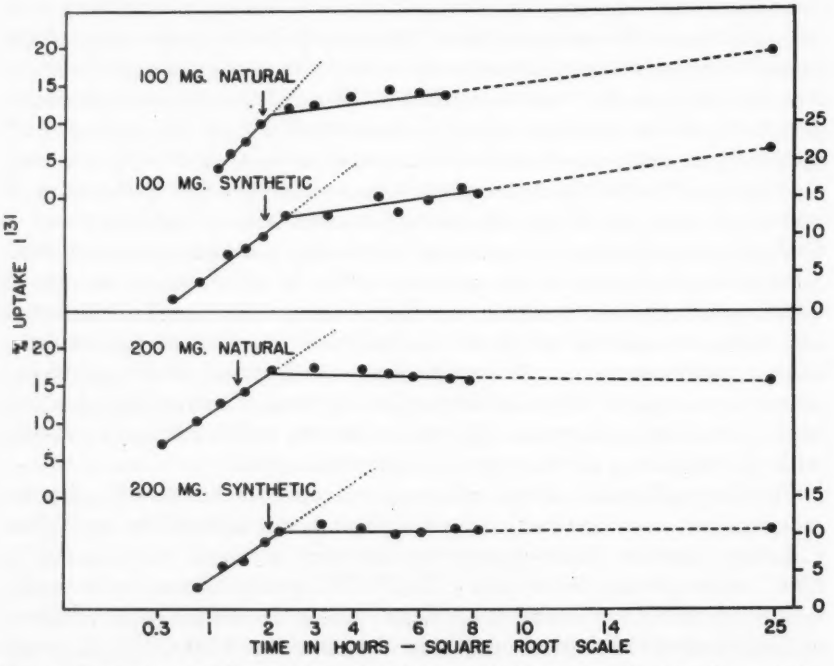


FIG. 2. Comparison of the antithyroid activity of the racemic and naturally occurring *l*-5-vinyl-2-thiooxazolidone in man. It can be seen that they possess identical potency.

thyroid activity (Fig. 2), being one-fifth as active as thiouracil in the rat and slightly more active than propylthiouracil in man. This compound has now been isolated from the seeds of rutabaga, white turnip, cabbage, kale, and rape. In addition, a spectroscopically similar substance has been detected in the seeds of Chinese cabbage, Brussels sprouts, broccoli, and kohlrabi. In fact, the only Brassica seeds in which the thiooxazolidone has not been found are those of cauliflower and mustard. It seems almost certain from the amounts of the material which have been isolated from the different seeds and their observed antithyroid activity when assayed whole, that this compound is the predominant if not the only goitrogen they contain.

The thiooxazolidone does not occur free in an active form in these plants. Heating or boiling the intact plant tissue destroys its goitrogenic activity, although once the seeds of turnips have been finely ground and allowed to steep in an aqueous solution a few minutes, the full activity is acquired and is not destroyed by prolonged boiling or evaporation. Grinding the untreated food with alcohol will also destroy its activity. This inactivation by heating or by alcohol is not accomplished by a destruction of the active principle. The heating apparently inactivates an enzyme which is essential for the liberation of the goitrogen from some combined, inactive form. The thiooxazolidone precursor is water soluble, and if an aqueous extract of ground, inactivated seeds is treated with a purified enzyme preparation from the seeds, the thiooxazolidone is formed in amounts calculated to be present from the known potency of the seed.

Just what the precursor might be is as yet unknown, but preliminary studies have indicated that the antithyroid agent may be present in the form of a glycoside. The aglycone component may exist as the thiooxazolidone itself or may be some straight chain compound, such as an isothiocyanate, which cyclizes when the glycoside linkage is enzymatically broken. Gastric contents do not appear to inactivate the enzyme which liberates this compound, for although only the unhydrolyzed precursor is present in chunks of rutabaga, these chunks possess marked antithyroid activity when eaten. The enzyme appears to be necessary for development of the activity of the food since the cooked vegetables do not depress the iodine uptake, although intact thiooxazolidone precursor is known to be contained in them.

Although the seeds of most of the Brassicae tested contained thiooxazolidone, the edible portions of none except rutabaga and white turnip yielded a detectable quantity of this compound. Several different varieties of cabbage head were assayed without avail. This, plus the fact that practically no antithyroid activity was found in cabbage by assay in either rat or man, makes cabbage goiter difficult to understand.

DISCUSSION

It is quite possible, of course, that the goiter produced by cabbage and the goiter produced by Brassica seeds and rutabaga are two entirely different entities. The fact that adding iodine to the diet will completely inhibit the development of cabbage-goiter whereas it will only partially inhibit Brassica seed-goiter indicates that such might be the case. Prolonged feeding of cabbage might lead to iodine deficiency which could easily be overcome by adding this element, whereas the Brassica seeds, as we now know, contain the antithyroid thiooxazolidone. It is very difficult to conceive that the goitrogenicity of cabbage might be due to some other mechanism, and it would indeed be strange if cabbage, which initiated the interest in Brassica-goiter and led to the isolation of an antithyroid compound from other members of this genus, did not contain the compound at all! This paradox might be partially explained by the finding that different varieties of the same species and even separate batches of the same variety grown in different localities or at different times have a marked variation in their content of thiooxazolidone. Thus the cabbage heads examined up to now may have been of a particularly impotent variety. Since in one assay of this vegetable on a human subject a positive antithyroid response was obtained, such an explanation may not be too unlikely.

It becomes pertinent at this point to attempt to relate the pathogenesis of certain cases of human goiter to the naturally occurring antithyroid compound described above. It must be admitted at the outset that in an overwhelming percentage of patients seen with goiter at this institution no clear dietary history of a high intake of these goitrogenic foods can be elicited. Certain goitrous individuals have been seen, however, who do consume a considerable amount of these vegetables. Two cases have been brought to our attention of individuals who developed goiters while eating cabbage. When the cabbage was omitted, the thyroids became smaller; and when it was given again, the glands resumed their growth (27).

The lack of adequate food during the recent war presented an opportunity to observe the influence of a chiefly vegetarian diet on large segments of the population. In the Low Countries, particularly, the main portion of the diet consisted of cabbage, turnip, or related foods. Several reports have appeared indicating that such eating habits were accompanied by an increased incidence of simple goiter.

Bastenie (28) reported that the incidence and severity of thyrotoxicosis in Belgium decreased during 1941–1944 as compared to 1936–1939. The incidence of simple goiter increased during the same period from 16 to 26 per cent. He believed this was caused by the high consumption of kohlrabi, cabbage, and similar vegetables. Schweitzer (29) found that there was a reduction of the basal metabolic rate in Holland from an average of plus

8 per cent in 1939 to an average of plus 2 per cent in 1943. This, too, was believed associated with the change in dietary habits. Himsworth (30) has told of a monastery in Belgium where the monks, during the war, were forced to subsist on a diet of "Swedes" (rutabagas) and tulip bulbs. Since the former are probably somewhat more edible than the latter, it is assumed that the rutabagas were consumed in greater quantity. All the monks developed goiters on this diet.

On the other hand, Meulengracht (31) and Grelland (32) report an increase in the incidence of thyrotoxicosis in Denmark and Norway during the war. In Denmark, the nutritional status remained satisfactory throughout the period of hostilities, and the incidence of hyperthyroidism remained at a high level until 1945. In Norway, however, although there was a marked spurt in the incidence of the disease in 1941, this had approached the prewar level by the following year. Since the decrease in the number of cases of Graves' disease occurred concomitantly with a worsening of the food situation, it is not unlikely that large amounts of turnip and related vegetables began to be consumed at that time, making conditions in Norway comparable to those in Belgium and Holland. Whether the above explanation for the variation in thyroid disease of these countries during the war is correct or not is, of course, subject to considerable debate.

Going back a few years into the literature, one finds that the clinical applicability of cabbage goiter was contested at that time, also. Wegelin in 1935 (33) stated that the people of southern and western Switzerland ate more cabbage and other vegetables than those in other parts of the country, where goiter was much more common. Suk (1931), however, found that the goitrous population of Carpathian Ruthenia lived on practically nothing but cabbage (34). Almost their only food from October through December was pickled or raw, stored cabbage.

Although it is unlikely that goiter will result if these goitrogenic foods are eaten in normal quantity, if, as sometimes happens, the dietary intake is limited almost entirely to turnips or similar vegetables, the amount of antithyroid material ingested may be sufficient to inhibit thyroxine synthesis and thereby cause thyroid enlargement.

One fact should be remembered. At least so far as the thiooxazolidone is concerned, cooking the vegetables either whole or in chunks before eating will prevent any goitrogenesis. No enzymes appear to exist in the body which are capable of forming this compound from its precursor. If the food is cut into small pieces or allowed to stand in water for a considerable period of time before cooking, however, enough hydrolysis may occur to allow considerable portions of the active compound to be formed.

Since thousands of people subsist almost entirely on a vegetarian diet, often with large amounts of raw vegetable juice, and very few of these

appear to have goiters, it is unlikely that the situation deserves becoming alarmed about. It is worth keeping in mind, though, that the sporadic goiter may occasionally be due to the ingestion of goitrogenic foods such as have been described. It is certainly not at all improbable that other naturally occurring goitrogens may in the future be isolated from other foods and that these may not require enzymatic liberation to acquire their antithyroid activity. Several vegetables and fruits, such as strawberries, pears, and carrots, were found to possess a certain degree of antithyroid activity in man (22) although no thiooxazolidone has yet been identified in them.

SUMMARY

The occurrence of a naturally occurring goitrogen of the thiocarbonamide series, *l*-5-vinyl-2-thiooxazolidone, in turnip and rutabaga root is reported. The possible relationship of this or similar compounds to the pathogenesis of certain cases of simple goiter is discussed. Although no definite conclusions can yet be reached, it seems probable that a high intake of certain of these goitrogenic vegetables by man may result in thyroid enlargement.

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ORGANIC AND INORGANIC IODINE: THEIR RECIPROCAL METABOLIC FATES*†

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A CHIEF function of the thyroid gland is to convert inorganic iodine (iodide) to a state of higher oxidation, equivalent to elementary iodine. When various animal proteins, except gelatine which is nearly devoid of tyrosine, are treated with elementary iodine, thyroxine is formed *in vitro*. In myxedema, the patient's own serum when combined with iodine (1) (2) will restore his health; whereas sodium iodide usually will not. A more striking demonstration is the relief of myxedema by the parenteral administration of elementary iodine (3) (4). The higher state of oxidation allows iodine to form the essential organic iodine complex (5) present in all tissues ("thyrenzyme," so-called). As this is degraded in the course of metabolism, it reverts to the inorganic form. Therefore, in broad terms, a large section of thyroid physiology may be described as the reciprocal metabolism of organic versus inorganic iodine.

It is the purpose of this communication to report observations on the mutual interconversion of organic and inorganic forms of iodine. Apart from its basic interest, an understanding of this topic is essential to avoid mistaken diagnosis through misinterpretation of clinical chemistry.

DEFINITIONS

To clarify the subsequent discussion, the following definitions and comments are pertinent:

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Thyroid hormone signifies any biologic material which reproduces the characteristic physiologic actions of the thyroid gland, *e.g.*, the relief of myxedema. The term includes thyroxine, thyroxine polypeptide, thyroglobulin (thyroid "colloid") and any other biologic material which has the characteristic endocrine potency of desiccated thyroid, U.S.P.

Protein-precipitable iodine signifies the iodine contained in a protein precipitate of serum or tissue extract obtained by any procedure whatsoever. Under favorable circumstances, the iodine so precipitated from blood serum or tissue extracts is a good index of thyroid function, whether the precipitant be heat (coagulation), alkaline zinc salts or acetone. Indeed, these procedures are commonly used by clinical chemists and endocrinologists in the analysis of blood in clinical diagnostic problems. Unfortunately, as will be discussed presently, the precipitable iodine may be derived in part from sources which have no endocrinologic significance.

"Hormonal" iodine is a term which signifies the iodine of the thyroid hormone in its natural physiologic forms in various tissues of the body. It may arise naturally from the physiologic activity of the thyroid gland itself; or it may arise from effective substitution therapy with desiccated thyroid, thyroxine or other medication. In the thyroid gland, only about one-fourth of the hormonal iodine exists as thyroxine, and this is not free, but combined in thyroglobulin. In the serum and in peripheral tissues, likewise, there is suggestive evidence (5) that thyroxine is bound to protein carriers and that it does not account for all the organic iodine. This last statement is disputed by some authorities; but until the facts become clearer, it is not justifiable to speak of "serum thyroxine iodine" as if it constituted the whole of the circulating natural iodine.

Serum organic iodine signifies the sum of the "hormonal" iodine plus organic iodine in other forms, such as the drugs used to visualize the gall bladder.

"Thyrenzyme" signifies the form in which thyroid hormone operates in peripheral tissues, *e.g.*, skeletal muscle. Just as heme is the active prosthetic group which combines with globin to form hemoglobin, so thyroxine in tissues combines with a cell constituent to form "thyrenzyme." Although this vague analogy with hemoglobin is a helpful concept, no implication is intended that the precise chemical union which anchors the prosthetic group is the same in the two cases.

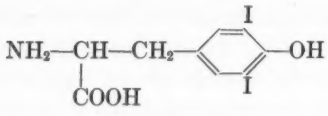
EXPERIMENTAL

The observations reported herewith concern inorganic iodine (iodide), as contrasted with organically bound iodine. The organic compounds studied were diiodotyrosine,[1] phenyl diiodohydroxy phenyl propionic acid

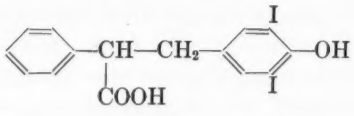
[1] For the diiodotyrosine and "Priodax" used in these studies the authors are indebted to Dr. Edward L. Henderson of Schering Corporation.

("Priodax"),[1] and phenyl diiodohydroxy cinnamic acid. Both stable iodine, I^{127}, and radioactive[2] iodine, I^{131}, were employed in these studies. The observations fall into two general categories; (a) observations on human material, mainly from cancerous patients, and (b) metabolic observations on rats.

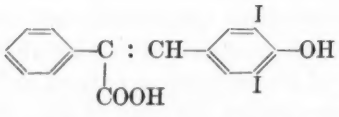
In following the fate of the compounds administered, special attention was paid to the binding of the organic material to protein. Special attention was paid also to the difference between true "hormonal" protein-bound iodine and "spurious" protein-bound iodine: because the latter has no endocrinologic significance. As a control substance for diiodotyrosine, phenyl diiodohydroxy phenyl propionic acid ("Priodax") was selected because it is not an amino acid and therefore not an amphoteric molecule. This feature is evident from the respective formulae, which show that in phenyl diiodohydroxy phenyl propionic acid ("Priodax") the amino group of diiodotyrosine has been replaced by a phenyl group.



Diiodotyrosine



Phenyl diiodohydroxy phenyl propionic acid ("Priodax")



Phenyl diiodohydroxy cinnamic acid

Preparation of radioactive diiodotyrosine

The radioactive diiodotyrosine was made from tyrosine by treatment with iodine in sodium hydroxide solution at approximately 2° C. The radioiodine was formed in a finely divided state by the reaction of iodide and iodate.

In a typical preparation, iodine was prepared by allowing 0.47 cc. of 1 N potassium iodide solution to react with 0.55 cc. of M/5 potassium iodate. Next, the radioiodide was added, followed by 5 drops of sulfuric acid (1–1 by volume). The supernatant liquid was removed by centrifuging, the iodine washed once with 1 cc. of water and centrifuged

[2] I^{131}, the radioactive iodine used in these investigations was obtained from the Oak Ridge National Laboratory, Carbide and Carbon Chemicals Corporation, Oak Ridge, Tennessee, through the Isotopes Division, Atomic Energy Commission.

again. The tube was then cooled in an ice bath. To it was added rapidly a cold solution made by dissolving 25 mg. of tyrosine in 0.4 cc. of 1 N sodium hydroxide cooled in an ice bath. The mixture was stirred, with cooling, until no further free iodine remained. A trace of sodium bisulfite was added, followed by 3 drops of acetic acid. After standing fifteen minutes in ice, the mother liquor was removed by centrifuging and the diiodotyrosine brought into solution in 1.5 cc. of water by 1 drop of sulfuric acid (1–1 by volume). This solution was centrifuged to remove a trace of amorphous matter, and the diiodotyrosine was reprecipitated by the addition of sodium acetate solution. The product was washed twice with 1 cc. of water by centrifuging, filtered and dried. The yield was 25 mg.

This yield *with respect to radioactivity* was essentially quantitative in relation to the proportion of diiodotyrosine obtained, as judged in terms of the total theoretical yield (65 mg.). Of course, iodine that is reduced to iodide and does not enter the molecule amounts to 50 per cent of the theoretical I_2 used.

Similarly, radioactive phenyl diiodohydroxy phenyl propionic acid ("Priodax") was made according to the following procedure, which was patterned after the synthesis of radioactive diiodotyrosine just described. For phenyl diiodohydroxy phenyl propionic acid ("Priodax") 33 milligrams of phenyl hydroxyphenyl propionic acid was used instead of 25 milligrams of tyrosine. The reaction was conducted at room temperature; and the product thereof was precipitated from 3.5 ml. of 5 per cent sodium bicarbonate solution by adding dilute acid.

Clinical observations

To supplement the excellent report of Keating and Albert (6), which appeared during the present experiments, human tracer clearances were obtained with radioiodide, I^{131}. These clearances included *pulmonary* clearance, which is of special interest in cases receiving therapeutic doses of isotope. On forced exhalation against or into an appropriate detector, the indicator needle on an ionization meter can be observed to rise abruptly. With a suitable apparatus, as shown in Figure 1, the exhaled radiation can be measured and related to the radioactivity of the blood.

Pulmonary clearance. The following example will illustrate the procedure. In a patient studied, 14 millicuries of radioactive iodine had been administered orally for the treatment of a thyroid cancer. Forty-eight hours after the ingestion of this medication, the total residual I^{131} was estimated at slightly less than 700 microcuries. At this stage the patient exhaled almost 2 microcuries per breath while his blood serum ran parallel in specific activity.

In industries employing radium and related substances, Hoecker (7) and others (8) have used the exhaled air to measure the extent of fixation of the radioactive material. The technique for radioiodine is essentially

similar, except that different mathematical constants and a somewhat different design of apparatus are employed.

"Spurious serum iodine" of nonthyroidal origin. In previous studies Salter (9) has emphasized the falsely high values, wrongly indicating hyperthyroidism, sometimes obtained in analyses of serum from normal patients or even hypothyroid patients. For instance, when normal individuals or euthyroid patients are given medicaments rich in iodine, the

FIG. 1. Schematic crude diagram of method for determining pulmonary clearance. More appropriate devices are being devised, including the substitution of an ionization chamber for the Geiger-Müller counter.

precipitated serum proteins will contain not merely genuine "hormonal" iodine but also an admixture of iodine derived from the iodine-containing drug. This feature is illustrated in Figure 2. Indeed, Salter (10) introduced the term "hormonal" for use only when the clinical chemist had taken reasonable precautions to exclude exogenous iodine compounds as a source of protein-precipitable serum iodine. Such technical precautions have been described in detail elsewhere (5). Earlier studies have included iodide in excess (11), the sodium salt of mono-iodo-methane sulfonic acid ("Skiodan") (12), thyroxine (13), and split products of thyroglobulin (9).

The present studies extend these observations to diiodotyrosine and to phenyl diiodohydroxy phenyl propionic acid ("Priodax").

The concentrations of organic iodine so produced were adjusted to correspond to the serum concentrations produced in rats in the metabolic studies to be described presently. Both protein-precipitable and non-precipitable iodine were measured; and both stable and radioactive isotopes were estimated. The general procedure was that described by Salter and Johnston (5) for the addition of thyroxine; but in addition, heat coagulation was compared with precipitation by cold acetone.

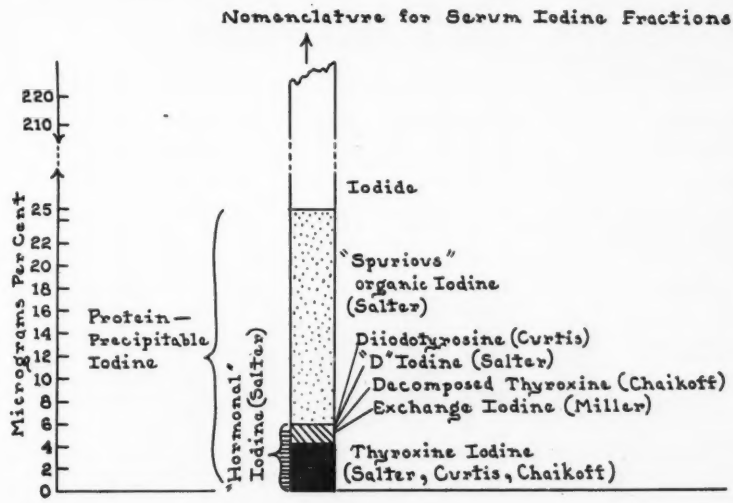


FIG. 2. This diagram indicates the sort of spurious elevation of protein-precipitable iodine under heavy iodide therapy, originally described by Salter (9) and recently elaborated by Swenson and Curtis (14) and by Danowski (15).

One such example will suffice. To 1.0 ml. of human serum containing 4.5 micrograms per cent of "hormonal" iodine and 0.6 micrograms per cent of iodide, were added 13.0 micrograms of phenyl diiodohydroxy phenyl propionic acid ("Priodax") in solution and then, with vigorous stirring, nine volumes of cold acetone containing dilute acetic acid. The flocculent precipitate was centrifuged down, washed with 90 per cent acetone, and again centrifuged. Iodine was then estimated in both the protein precipitate and in the supernatant fluid. The results were 5.5 and 12.4 micrograms per cent respectively. On heat-coagulation, however, the precipitate showed 9.9 micrograms per cent.

Observations on human subjects. In Table 1 are presented clearances of

TABLE 1. HUMAN PULMONARY CLEARANCE OF I^{131}

Patient	Days after oral dose	Radioactivity of 1 ml. serum		Expired air (counts per minute)*	Daily urinary excretion		
		Per cent of dose	curies $\times 10^{-9}$		I^{131} (per cent of dose)	I^{127} (μg.)	Urine volume (ml.)
Ja.							
N. H. H. #C15816							
Test dose	0.0						
(150 μc.)	1.5	6.9×10^{-3}	13	6,700			
	2.5	—	—	2,300			
	3.8	1.7×10^{-3}	3.1	950			
Therapeutic dose	0.0						
(45.8 mc.)	1.0	82×10^{-5}	423	Off scale	35 (18 hrs.)	41	1,590
Male, 63 yrs.	1.75	35×10^{-5}	159	Off scale	36	95	3,820
83.6 Kg.	3.75	30×10^{-5}	138	Off scale	7.7	225	3,160
B.M.R. = −45 per cent	6.0	—	—	10,815	2.6	170	1,890
Thyroid carcinoma, metastatic in manubrium sterni, treated with I^{131}.	6.8	5.6×10^{-5}	29	10,065			
Serum "hormonal" iodine = 0.9 μg. %	10.75	3.2×10^{-5}	15	3,710			
Serum iodide = 1.5 μg.%							
Li.							
N. H. H. #49683							
Test dose	0.0						
(140 μc.)	1.3	—	—	210	48	89	810
	1.8	2.1×10^{-3}	4.0	—	2.6	130	1,120
Male, 60 yrs.							
60.1 Kg.							
Anaplastic cervical carcinoma							
Serum "hormonal" iodine = 4.1 μg.%							
Serum iodide = 1.6 μg.%							
Be.							
N. H. H. #B8364							
Test dose	0.0						
(150 μc.)	3.9	0.44×10^{-3}	0.94	62	—		
Therapeutic dose	0.0						
(45.8 mc.)	0.8	17×10^{-5}	80	Off scale	50	205	3,610
	1.8	12×10^{-5}	56	Off scale	19.8	206	3,440
Female, 30 yrs.		—	—	23,000 –	2.5	138	3,380
53.2 Kg.	3.8	8.6×10^{-5}	40	10,300	0.75	55	2,980
B.M.R. = −30 per cent	4.8	—	—	6,000			
	5.8	5.0×10^{-5}	23	4,400			
	7.0	—	—	4,050			
Recurrent thyroid carcinoma, treated with I^{131}	17.0	0.63×10^{-5}	2.9	410			
Serum "hormonal" iodine = 2.1 μg.%							
Serum iodide = 5.2 μg.%							
Ma.							
N. H. H. #C27671							
Test dose	0.0	—	—	—		—	—
(147 μc.)	1.0	13×10^{-3}	18.7	2,500		—	—
Female, 29 yrs.							
54.1 Kg.							
B.M.R. = +1 per cent							
Thyroid carcinoma with lateral node metastasis							
Serum "hormonal" iodine = 4.6 μg.%							
Serum iodide = 1.9 μg.%							

* Readings taken with Tracerlab, Inc. (Boston) Model SU-3A laboratory monitor equipped with Geiger tube TGC-1 through probe window 2.8 cm. in diameter.

tracer radioiodide for several patients at both the "test-dose" and the "therapeutic-dose" levels. The pulmonary clearance is given in detail for patients Ja and Be, who suffered from thyroid cancer. Both patients were ambulatory and active, although the first was being treated for a metastasis in the manubrium sterni and the latter for the thrice-recurrent growth of a thyroid adenopapilloma, still confined to the cervical region. The

serum iodide of these patients was approximately 0.5 micrograms per cent and their daily urinary excretion of iodide between 150 and 250 micrograms per diem. The unit of radioactivity used in Table 1 is the older Oak Ridge curie, which was 1.75 times the present Oak Ridge curie.

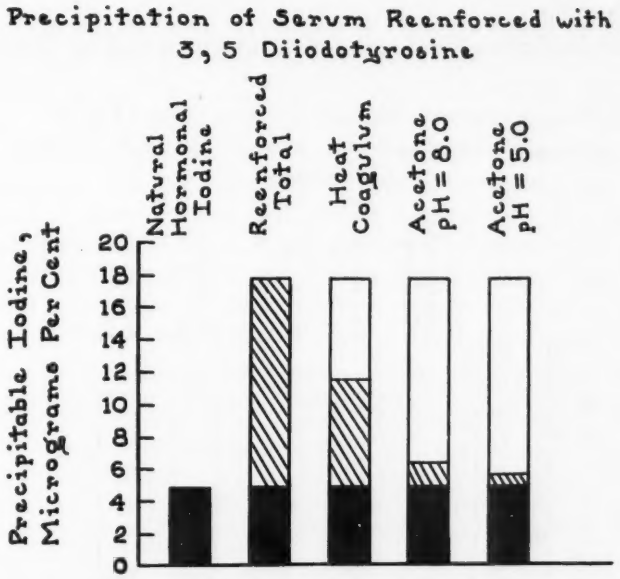


FIG. 3. When serum containing diiodotyrosine is precipitated by heat-coagulation a spuriously high value for protein-precipitable iodine is obtained. This error can be obviated largely by precipitating with acid-acetone.

The pulmonary clearance suggests that nearly 0.3 per cent of the body's reserve of iodide was exhaled per hour. Therefore, in the course of three or four weeks over 1 per cent of an assimilated dose might be lost by expiration, on the assumption that the loss by sweat was negligible. In extremely hot weather doubtless less would be exhaled proportionately, because the relative loss of water through the lungs would be less. This relation of iodide in expired water vapor to the iodide reserve in body water may afford a useful gauge of dosage during therapy with radioiodide.

The results just cited hold only when test or tracer doses are employed. When appreciable amounts of carrier iodide were also given, the loss of radioactive iodide by the urine was greater and less was trapped in the thyroid.

Organic iodine in human serum. When iodide is added to human serum in excess, as shown by Bassett, Coons and Salter (11), the heat-coagulated

proteins contain an excess of "spurious" iodide. When the proteins are precipitated with cold acetone, as described by Salter and Johnston (5), however, this iodide is largely removed, provided that the coagulum is properly washed. In so doing, care must be taken, as demonstrated by Swenson and Curtis (14), to avoid splitting off true "hormonal" iodine from the protein.

As shown in Figures 3 and 4, the same principles hold for diiodotyrosine

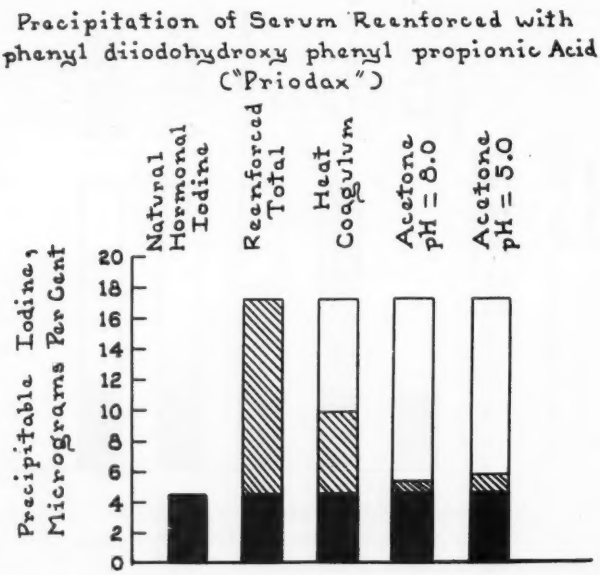


FIG. 4. When serum containing phenyl diiodohydroxy phenyl propionic acid ("Priodax") is precipitated by heat-coagulation, a spuriously high value for protein-precipitable iodine is obtained. This error can be obviated largely by precipitating with acetone.

and for phenyl diiodohydroxy phenyl propionic acid ("Priodax"). On heat coagulation, the amino-acid diiodotyrosine is heavily absorbed by the protein. Even with acetone precipitation this will occur to some extent unless the initial pH is approximately isoelectric, because the amino acid is amphoteric. With phenyl diiodohydroxy phenyl propionic acid ("Priodax") the danger is less, because it lacks the basic amino group (*see* Fig. 4). Therefore, although the complex "protein-Priodax" can occur in acid medium, the converse complex "Priodax-protein" does not occur in alkaline medium. This is illustrated in Figures 3 and 4. Of course, all protein precipitates must be washed adequately, else the iodine-containing mother liquor will confuse the separation.

Metabolic experiments (rats)

Methods

The metabolic fates of iodide, diiodotyrosine, phenyl diiodohydroxy phenyl propionic acid ("Priodax") and phenyl diiodohydroxy cinnamic acid (called P-76) were studied in adult male Wistar rats, weighing nearly 300 grams each. Both stable and radioactive forms of these substances were used. The material was injected intraperitoneally in physiologic saline solution. The dosage was adjusted in each case to yield 500 micrograms of total iodine per 100 grams of rat. The animals were fasted overnight before the injection. They were sacrificed at increasing intervals after the injection, so that a composite curve of iodine concentration could be attained. In order to conserve the brain, each animal was rapidly reduced to the beginning of Stage II anesthesia with diethyl ether and then exsanguinated by carotid bleeding. Care was taken not to injure the thyroid gland. Various organs were then removed as rapidly as possible, weighed and chilled in an ice box at 4° C. The blood was treated similarly.

From each organ approximately 900 milligrams was taken and weighed. This sample was finely ground with a miniature conical mortar and pestle in order to homogenize it. Distilled water, made alkaline with ammonia to a concentration of 0.015 normal, was used to dissolve the protoplasmic constituents. The final homogenate had a volume of about 6 ml., which was collected in a 15 ml. conical centrifuge tube. With I N acetic acid the turbid solution or suspension was brought to the isoelectric point near pH 5.0, and the centrifuge tube was heated to about 95°C. in a boiling water bath. As the protein coagulated it was stirred intermittently to prevent the formation of large masses of coagulum. After standing ten minutes at 95°C., the tube was centrifuged and the supernatant liquor saved. The coagulum was washed once with 2 ml. of water and centrifuged again.

The combined supernatant liquors were divided. Half was used to measure radioactivity and half to measure stable iodine. In the latter instance, the iodine extractable with butyl alcohol was separated from the hydrophilic iodide. The heat-coagulum was dissolved in 1 ml. of warm I N sodium hydroxide.

The measurements of radioactivity were made according to the method described by Salter (16, 17) in which the unknown, X, is compared with X+a, where "a" is a known aliquot of the original radioactive material. This method obviates the need for ashing the sample and at the same time compensates for various geometric factors and errors due to internal absorption or adsorption. Stable I^{127} was measured by the procedure described in detail by Salter and Johnston (5).

As an alternate method to heat coagulation near pH 5.0, certain homogenates were also precipitated with cold acetone at pH 5.0, as described by Salter and Johnston (5).

Because the measurements of radioactivity were detailed and laborious, comparative measurements were made at a time when the distribution of iodine had become somewhat stable. This appeared to be most illuminating three hours after injection, but probably it would be interesting to carry out the detailed analysis at longer intervals. At any rate, the detailed

TABLE 2. RADIOACTIVE DIIODOTYROSINE IN RAT (±300 GM.), THREE HOURS AFTER INJECTION OF 500 μg. I* PER 100 GM.

Precipitant	Organ	Weight of organ (Gm.)	Stable iodine, I^{127} μg.%	Labelled iodine, I^{131} μg.%	Precipitant	Weight of organ (Gm.)	Stable iodine, I^{127} μg.%	Stable iodine, I^{127} μg.%
Heat-coagulation	Thyroid	0.0174	21,400**	1,440**	Acetone	0.0198	8,500	Thiouracil-treated 246
			2,850	3,410			69,500	2,384
			24,250	4,850			78,000	2,630
	Heart	1.19	9	210		1.090	20	—
			204	121			1,540	
			213	331			1,560	
	Kidneys	2.02	62	114		2.2778	81	37
			103	318			190	64
			165	432			271	101
	Serum	—	159	110		—	26	13
			150	865			452	28
			309	975			478	41
	Lung	1.84	113	46		4.44	21	—
			26	592			263	
			139	638			284	
	Muscle	—	28	22		—	8.1	18
			26	164			127.	15
			54	186			135.	33
	Testes	2.65	17	21		2.910	6.4	—
			39	251			197.	
			56	272			203.	
	Brain	1.73	2	2		2.1224	13	—
			14	17			58	
			16	19			71	
	Spleen	0.99	66	1		0.7860	9.4	10
			70	100			153.	31
			136	101			162.	41
	Liver	9.31	74	18		8.940	24	20
			76	1,604			52	22
			150	1,622			76	42

Acetone	Normal thyroid		0 hr.	3 hr.	8 hr.	24 hr.	48 hr.	72 hr.
		Weight (Gm.)	0.015	.0198	0.0149	0.0188	0.0208	0.0152
		Iodine concentration, μg%	14,500	8,500	9,480	18,700	10,900	16,600
			1,500	69,500	73,600	66,800	75,700	82,200
			16,000	78,000	83,080	85,500	86,600	98,800

* Isotope, whether radioactive or stable.

** The upper figures indicate "protein-bound" iodine; the middle ones, "soluble" iodine; and the lowest ones, the "total" iodine.

As reported elsewhere, by the third hour the "soluble" iodine is over half inorganic.

TABLE 3. TISSUE DISTRIBUTION OF IODINE FROM PHENYL DIIODOHYDROXY PHENYL PROPIONIC ACID ("PRIODAX") AND PHENYL DIIODOHYDROXY CINNAMIC ACID (after intraperitoneal administration in a dose equivalent to 500 micrograms of iodine per 100 grams of rat)*

Tissue	Micrograms of iodine per 100 grams of tissue								
	Hours after injection								
	2	3	4	8.5	12	25	36	57	72
"Priodax"									
Liver	35** 32 — 67				34 26 — 60	32 18 — 50		16 5 — 21	14 —
Kidney	24 08 — 32				21 13 — 34	16 14 — 30		11 7 — 18	8 —
Muscle	19 13 — 32				13 10 — 23	9 7 — 16		9 3 — 12	8 —
Serum	13 21 — 34				12 4 — 16	12 (4) — (16)		9 6 — 15	7 —
Thyroid	4,220 14,680 — 18,900				4,020 6,180 — 10,200	3,840 4,560 — 8,400		3,690 2,520 — 6,210	3,190 —
Spleen	14 21 — 35				15 5 — 20	15 6 — 21		— 15.2	15 —
Heart	24 7 — 31					19 13 — 32		8 9 — 17	
"Priodax" after thiouracil					Phenyl diiodo-hydroxy cinnamic acid, in normal rat				
Liver		7.8 31. — 39.	7.9 22. — 30.	32 10 — 42		17 25 — 42	40 —		
Kidney		24 —	12 26 — 38	17 13 — 30		11 6.8 — 18.	9.4 19. — 28.		
Muscle		8.1 —		11 18 — 29		— —	— —		
Serum		3.0 —	3.5 18. — 22.	7.6 19. — 27.		5.2 —	5.6 36. — 42.		
Thyroid		240 351 — 591	151 488 — 639	171 711 — 882		6,740 7,200 — 13,940	251 7,690 — 7,940		
Spleen		8.3 24. — 32.		13 34 — 47		8.8 8.5 — 17.	22 22 — 44		
Heart						9.7 11. — 21.	13 11 — 24		

* Male Wistar rats; average body weight 356 Gm. ± 15.

** The upper figures indicate "protein-bound" iodine; the middle "ones, soluble" iodine (by acetone treatment); and the lowest ones, the "total" iodine.

As reported elsewhere, by the third hour the "soluble" iodine is over half inorganic.

radioactivities correspond to and coincide with measurements of stable iodine made in companion experiments.

Results

Tables 2 and 3 show the concentrations found in various organs at progressive times for diiodotyrosine and phenyl diiodohydroxy phenyl propionic acid ("Priodax") (18) (19). For comparison, a similar series for iodide is given in Table 4.

TABLE 4. RELATIVE DISTRIBUTION OF RADIOIODIDE THREE HOURS AFTER 7 μc. I^{131} GIVEN INTRAPERITONEALLY TO RAT ($\pm$300 GM.)

Tissue	I^{131} counts per minute %	I^{127} micrograms %
Liver	1,690	3.3
Kidney	1,700	2.0
Serum	736	3.0
Muscle	803	2.0
Thyroid	324,000	141,000
Spleen	705	2.2
Heart	322	2.2
Brain	437	2.7

Of special interest is the concentrating activity of the liver and kidney in the case of the organic compounds. Table 3 shows that the hepatic concentration of phenyl diiodohydroxy phenyl propionic acid ("Priodax") predominated; whereas Table 2 shows that for diiodotyrosine the concentration was also high in the kidney. From time-curves constructed with these data, shown in Figures 5 and 6, it was determined that at the end of three hours a moderately stable equilibrium had been established in the organism. Therefore, this interval was selected as an appropriate time to study the partition of iodine between organic and inorganic forms within the various types of tissues.

Organic vs. inorganic iodine. Both heat coagulation and acetone-precipitation were used to precipitate the tissue proteins from their respective extracts. At the moment, the results obtained with heat coagulation will be stressed. To illustrate the experiment, detailed observations on Rat No. 5 will be cited.

This animal was a male weighing 290 grams. After fasting overnight, 1.45 ml. of saline containing 1.45 mg. of radioactive diiodotyrosine was injected intraperitoneally. Three hours later the animal was sacrificed.

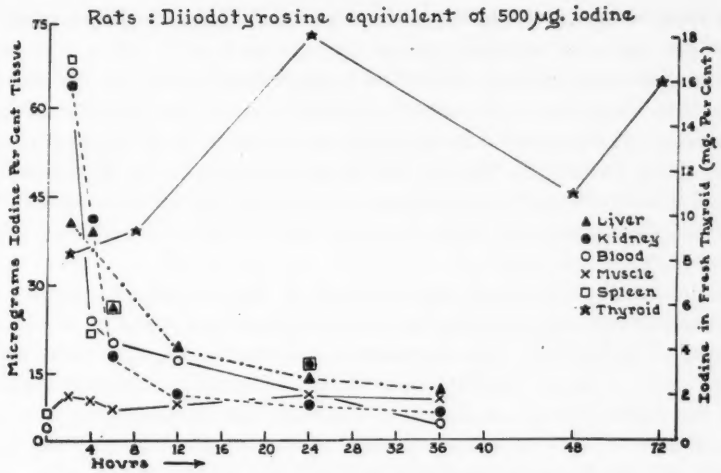


FIG. 5. Protein-bound iodine (by acetone precipitation) in various organs of rats following the injection intraperitoneally of diiodotyrosine. The dose of the drug contained 500 micrograms of iodine per 100 grams of rat.

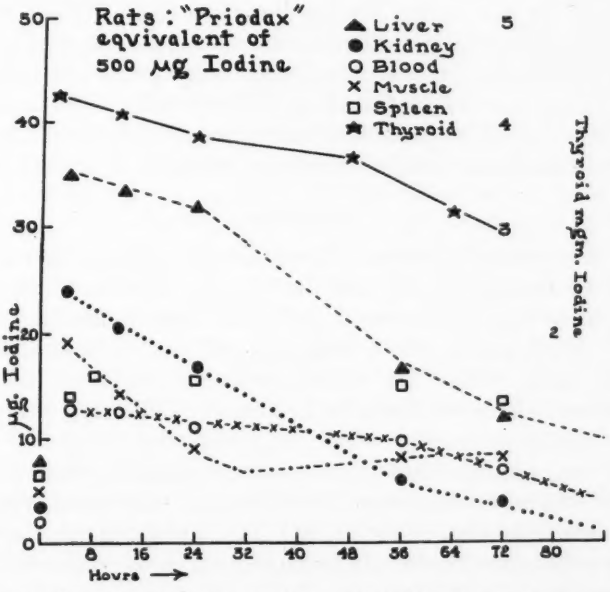


FIG. 6. Protein-bound iodine (by acetone precipitation) in various organs of rats following the injection intraperitoneally of phenyl diiodohydroxy phenyl propionic acid ("Priodax"). The dose of drug contained 500 micrograms of iodine per 100 grams of rat. The ordinates express iodine per 100 grams of tissue, as in Figure 5.

The fresh weights of the organs are given in Table 2. From each organ some 800 mg. was sampled and an extract made with dilute aqueous ammonia. This extract was subjected to heat-coagulation at the isoelectric zone. The coagulum was washed with water, and the supernatant liquors combined. The protein was dissolved in 1.0 cc. of 1 N sodium hydroxide in a boiling water bath. Half of the material was taken to determine radioactivity. Likewise half of the supernatant liquor was used for radioactivity, after alkalinization with sodium hydroxide to pH 9. In addition, acetone-precipitation was tried.

Radioactivity. In certain experiments it was possible to measure both radioactive and stable iodine. In general, these two types of measurement compared rather well. This agreement was due to the fact that the drugs constituted "carrier" rather than "tracer" material. A possible exception was the thyroid itself, as shown in Table 2. The data suggest that diiodotyrosine *per se* did not enter the parenchymal cells, but only the labelled iodide arising by decomposition of the drug.

Effect of thiouracil and of thiocyanate. To test this hypothesis further, a similar dose of radioactive diiodotyrosine was administered to rats poisoned with thiouracil. These animals had eaten a dog chow containing 1 per cent of thiouracil for six weeks. During this time their thyroid weights had increased fourfold to nearly 60 milligrams in weight. As shown in Table 2, the protein-bound iodine of their glands was not elevated significantly, although some increase in bound iodide occurred. As a contrast to this observation, animals poisoned with thiocyanate were studied. Such animals showed no significant uptake of radioactive iodine in any form.

DISCUSSION

There are two great reserves of organically bound iodine in the body, *i.e.*, that in skeletal muscle and that in the thyroid. These are not far from equal in their total amount, although one of them is bound in the relatively small gland, which comprises only one five-hundredth of the total body mass. One of the earliest studies of the fate of labelled iodide in the thyroid gland was made by LeBlond and Süe (20), who pointed out that the change in specific activity of the several iodine fractions within the gland was an important feature in measuring the progressive series of synthetic stages which occurs there. Several subsequent studies of this phenomenon have been reported (21) (22). Eventually the labelled iodine becomes apparent in the blood plasma protein, as the radioactive thyroxine or its derivative is secreted into the blood stream from the gland. The arrival of the catalyst within peripheral tissues can be followed through the tracer. Salter, Johnston and Gemmel (23) showed that in the course of two or three days it reached a maximum and then proceeded to decline

as it was used up and replaced with "thyrenzyme" made from stable iodide.

In this fashion the rate of degradation of the peripheral catalyst can be measured. As pointed out in Table 2, the building up of this material is dependent upon thyroidal action. In the thyroidectomized rat or thiouracil-poisoned rat, the administration of diiodotyrosine can not speed up the accumulation of thyroxine (24) or "thyrenzyme" in the peripheral tissues. Neither can the administration of diiodotyrosine speed up the formation of "thyrenzyme" by the *normal* rat. Normally the thyroid gland must start with iodide and laboriously form its organic compounds by itself. If radioactive thyroxine can be administered, however, it is picked up rapidly by the peripheral tissues and incorporated into protoplasmic protein, although a major portion is decomposed before it can be fixed.

Of course, the iodide reserve is subject to constant leakage through several routes. Of these avenues, thyroid clearance and renal clearance are the more important. If the specific radioactivity of the iodide reserve is high, however, even exhaled air may be measured for inherent radioactivity. As shown in Table 1 the concentration of radioiodide in body fluids may be estimated under appropriate conditions from the radioactivity of the breath.

Fate of exogenous iodine compounds. Ordinarily the organic iodine compounds found in the organs are of natural origin. From the standpoint of the peripheral tissues like muscle or brain, however, this distinction is of minor importance; and the thyroid gland is in effect an exogenous source. When diiodotyrosine prepared with stable iodine is administered, as shown in Table 2, there is an initial surge of the material into the blood stream and later into the tissues. The same experiment may be done with diiodotyrosine which is labelled. When this procedure is followed, as shown in Table 2, the very early liberation of iodine in the form of labelled radioiodide is evident. Thus the fate of diiodotyrosine soon becomes that of two substances, *i.e.* (a) of the parent substance as given, and (b) of the iodide which is split off. It may be observed that derivatives of diiodotyrosine such as phenyl diiodohydroxy phenyl propionic acid ("Priodax") behave rather similarly, although possibly the rate of decomposition of the compound into iodide is somewhat slower.

In interpreting these facts it must be born in mind that certain complicating features attend the administration of these compounds. A few of these have been investigated. As shown in Figures 5 and 6 there is a definite concentration of diiodotyrosine and its derivatives in the hepatic tissue. Later the material appears in the bile in concentrated form and may be absorbed from the intestine to complete a second cycle of metabolism. Also, as shown in Figures 5 and 6, the same thing may be said in respect

to renal concentration, which is incident to the tubular secretion of these materials. Simultaneously, of course, both organs are dealing with the iodide which has been split off; but the kidney effects no such differential concentration of iodide as in the case of the organic forms.

"Spurious" increases of protein-bound iodine from exogenous sources. The philosophy behind the term "hormonal" iodine (9) is that a well trained biochemist or clinical chemist has surveyed the results and has certified (as it were) that the result is not "spurious." It is possible in most cases to exclude these "spurious" high values, provided that the clinical chemistry is in the hands of a well trained individual.

It would appear that there are several possible types of "spurious" increase in the protein-bound iodine; and these must be understood by the clinical chemist if he is to avoid diagnostic confusion. Among these types are:

(i) Mechanical occlusion of iodide or of an organic iodine compound in the protein coagulum.

(ii) Adsorption of iodide ions on the protein.

(iii) Salt-formation of protein with the basic or acidic groups of an organic compound containing iodine.

(iv) Incorporation of exogenous iodine into the serum protein by oxidase action *in vitro* in shed blood, or *in vivo* (15).

(v) Contamination of the serum in the course of sampling or analysis.

(vi) Moreover, exogenous iodine in serum may be the result of administration by various routes such as by the cerebrospinal fluid or after cutaneous or vaginal application. Iodine from these sources may persist for weeks.

As indicated above, in many cases these difficulties can be avoided by using a suitable method for precipitating the protein. If the concentration of exogenous iodine compound is overwhelming, the problem can be simplified further by a preliminary overnight dialysis of the serum against cold distilled water. For this purpose a small cellophane sausage-casing is useful. Probably the same methods can be used for tissue extracts, with slight modifications.

SUMMARY

The mutual interconversion of iodide and organic iodine in the organism has been studied in man and in rats. Both stable and radioactive compounds have been employed. The organic compounds used were derivatives of diiodotyrosine, including phenyl diiodohydroxy phenyl propionic acid ("Priodax"). With all these organic compounds a prompt and progressive liberation of iodide complicates their metabolic history. Such sources of exogenous iodine lead to "spurious" increases in the protein-bound iodine of the serum, so that clinical diagnostic confusion may result

because the reported value is not truly "hormonal," *i.e.*, of thyroid origin. Through suitable precautions such complications usually can be circumvented.

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THE PHYSIOLOGIC ACTIVITY OF TETRABROMTHYRONINE AND TETRACHLORTHYRONINE*

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METABOLIC equilibrium in the body, as far as thyroid hormone is concerned, is maintained by the balance between two actions of this hormone, namely, its effects in stimulating the metabolism of tissues on the one hand and in depressing the function of the thyroid gland on the other. The latter effect is brought about in two ways: a) indirectly, by depressing the output of thyrotropic hormone from the pituitary gland, and b) directly, by depressing the activity of the thyroid follicles (Cortell and Rawson, (1)).

Several investigators have already utilized this property of thyroid hormone to depress the overactive thyroid of Graves' disease. Unfortunately, the high calorigenic action of the hormone makes its use in large dosage over a long period of time hazardous. The ideal compound for the control of the overactive thyroid would be one which retained the depressant effects on the thyroid and anterior pituitary gland shown by thyroxine, while having lost the property of stimulating tissue metabolism. The use of one such compound became possible, when, during our reinvestigation of the calorigenic activity of the optical isomers of thyroxine, it was found that most of the activity of racemic thyroxine resides in the laevo compound (Fig. 1). The activity of *d*-thyroxine, as shown in Figure 2, is relatively low, namely, about one tenth that of *l*-thyroxine. It was then suggested by Dr. Means that we use *d*-thyroxine to treat patients with Graves' disease. Unfortunately, the supply of *d*-thyroxine was limited and the problem could not be pursued further.

It then appeared that the desired compound might reasonably be sought among analogues of thyroxine, and as a first step it was therefore decided to examine the physiologic action of tetrabrom- and of tetrachlorthyronine, substances identical with thyroxine except for the replacement of iodine by bromine and chlorine respectively. Samples of both these compounds were synthesized according to the method of Schuegraf (2). It has already been shown by Gaddum (3) that tetrabromthyronine has the same qualita-

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tive effect in rats as thyroxine, although in much lower degree (*cf.* Le Blond and Grad, (4)). Also, both compounds have been found by Parkes (5) to stimulate metamorphosis in Xenopus tadpoles. The purpose of this paper is to report on the metabolic and clinical effects of these two substances in human myxedema.

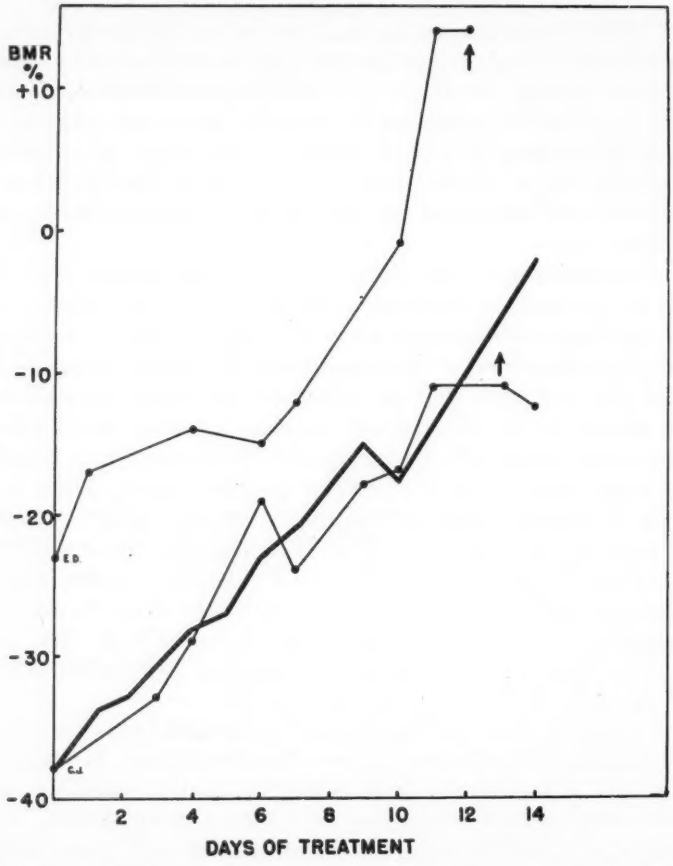

FIG. 1. The calorigenic response of 2 patients with spontaneous myxedema (E.D. and C.F.) to daily intravenous doses of *l*-thyroxine containing 0.25 mg. of iodine, shown in light lines. The heavy curve is the standard response to daily doses of 0.5 mg. of iodine in the form of thyroxine polypeptide. Small arrows indicate day of final dose.

METHOD AND MATERIALS

Each compound was assayed in patients with spontaneous myxedema, the bromine compound in 2 cases and the chlorine compound in 3 cases.

The material was administered daily intravenously and the resulting metabolic changes were compared with our standard assays of thyroxine and thyroxine-polypeptide. In assaying each compound, the initial dose was small. It was increased every two to four days, depending on the metabolic and clinical response. An attempt was made to adjust the dosage so as to approximate the metabolic response to our standard thyroxine polypeptide, given in daily dosage of 0.5 mg. of iodine.

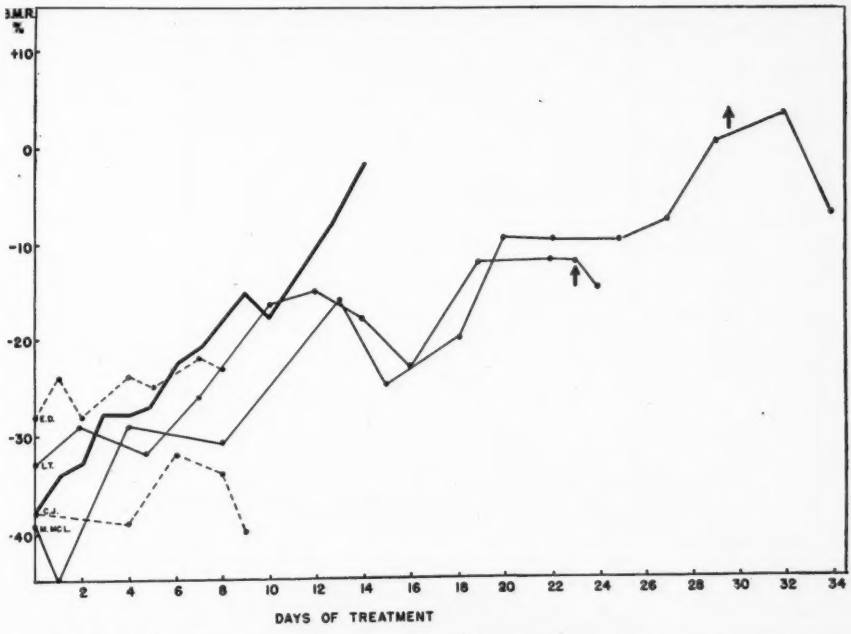

FIG. 2. The calorigenic response of patients with myxedema to *d*-thyroxine. The interrupted curves represent the response to daily intravenous doses containing 0.5 mg. of iodine. The two light curves represent the response to daily intravenous doses containing 1 mg. of iodine. The heavy line is the same standard as in the previous figure. The small arrows indicate day of final dose.

The first patient receiving tetrabromthyronine (M. S.) was started on 1 mg. daily. The dose was gradually increased to 15 mg. daily by the ninth day. The second patient (J. J.) received 10 mg. daily throughout the experimental period. The first two patients receiving tetrachlorthyronine (R. M. and H. C.) were started on 10 mg. daily and gradually increased to 80 mg. on the twentieth and twenty-third days respectively. The third patient was started on 100 mg. daily and increased to 125 mg. on the seventh day.

In addition to frequent basal metabolic rate determinations, one or more determinations of blood cholesterol and protein-bound iodine were made in each patient before, during and at the end of treatment.

RESULTS

The results of assay of tetrabromthyronine are shown in Fig. 3. It is obvious that a daily dose of about 10 mg. of this compound produces a

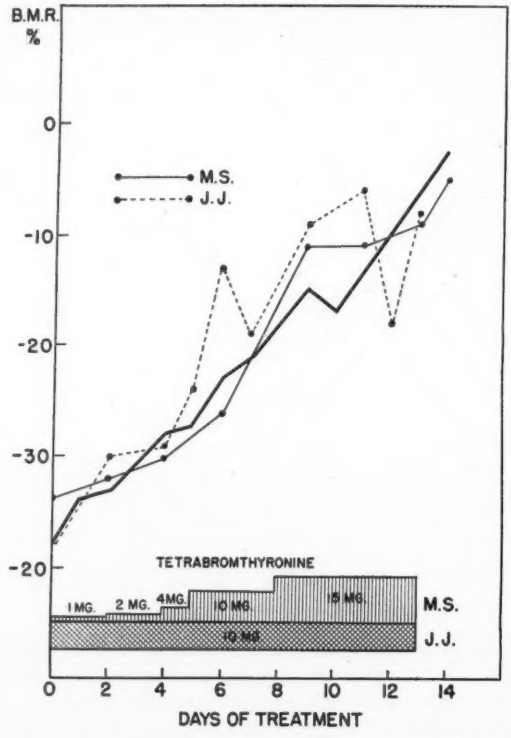

FIG. 3. The calorigenic response of 2 patients with myxedema to tetrabromthyronine. One patient (M.S.) received increasing amounts, starting with 1 mg., the average dose being about 10 mg. daily. The other patient (J.J.) received 10 mg. daily. The heavy line is the same standard as in the previous figures.

metabolic change equal to that of 1 mg. of thyroxine polypeptide (0.5 mg. I_2 or 0.75 mg. of thyroxine). In each case there was corresponding clinical improvement and the blood cholesterol dropped from 321 mg. to 121 mg. per cent in patient M. S.; and from 316 mg. to 190 mg. per cent in patient J. J. These data indicate that thyroxine by weight, is about

thirteen times as active as tetrabromthyronine, but taking into consideration the difference in molecular weight between thyroxine and tetrabromthyronine, then the former is about seventeen times as active.

Figure 4 shows the results of assay of tetrachlorthyronine. The first two

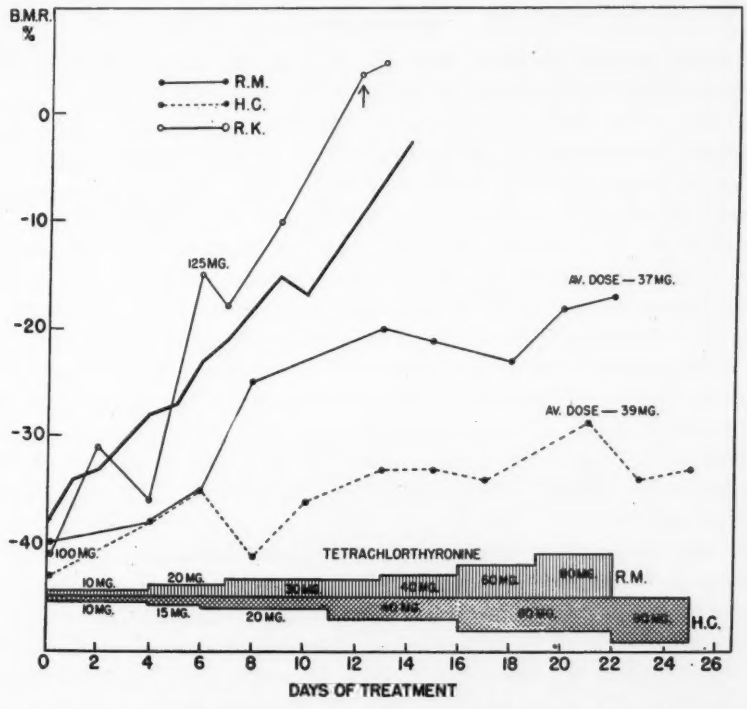

FIG. 4. The calorigenic response of 3 patients with myxedema to tetrachlorthyronine. Patient R.M. received an average dose of 37 mg. daily; patient H. C., 39 mg. daily; and patient R. K. started with 100 mg. daily and increased to 125 mg. daily on the seventh day. Small arrow indicates day of final dose. The heavy line is the same standard as in the previous figures.

patients, R. M. and H. C., received a daily dose averaging 37 mg. and 39 mg. respectively. The metabolic responses were definitely substandard. There was no doubt about the clinical response; and the blood cholesterol in the first patient dropped from 532 mg. to 245 mg. per cent, and in the second patient from 417 mg. to 245 mg. per cent. The third patient (R. K.) made a very good response. While on 100 mg. of tetrachlorthyronine daily for six days, the response seemed to equal that of thyroxine polypeptide, and on 125 mg. daily for the remaining seven days it seemed to exceed it.

The blood cholesterol dropped from 291 mg. to 167 mg. per cent. One may conclude that 100 mg. of tetrachlorthyronine produces the same caloric effect as 1 mg. of thyroxine polypeptide or 0.75 mg. of thyroxine. In other words, thyroxine, by weight, is about 133 times as active as tetrachlorthyronine and about 250 times as active in terms of molar equivalents.

DISCUSSION

It has long been assumed that the physiologic action of thyroxine is dependent upon the presence of iodine in the thyronine nucleus. That this is not the case is shown by the above results—the bromine and chlorine analogues of thyroxine qualitatively possess the same physiologic properties as thyroxine. It may be argued that iodine, in some way, replaces bromine or chlorine in vivo when tetrabrom- or tetrachlorthyronine is administered. If this were the case, then one would expect the levels of blood iodine to increase as the metabolism rose. Actually, the protein-bound iodine in the blood of these patients, already low, remained the same or decreased slightly. Of the 2 patients receiving tetrabromthyronine, the protein-bound iodine in patient M. S. dropped from 0.7γ to 0.3γ per cent; and in patient J. J. it dropped from 0.5γ to 0.1γ per cent. With the administration of tetrachlorthyronine, the protein-bound iodine of patient R. M. rose from 1.0γ to 1.7γ per cent, but in patient H. C. it dropped from 1.1γ to 0.7γ per cent, and in patient R. K. from 1.1γ to 0.5γ per cent.

As indicated at the beginning of this paper, the major interest in this problem is to determine whether the thyroid-depressant and calorigenic effects of the compounds tested bear the same quantitative relationship to one another as in thyroxine or whether one or another of these effects is disproportionately increased. Very little information is as yet available on this point. The work of Richards *et al.* (6) indicates that the depressing effect of tetrabromthyronine on the pituitary of rats is of the same order of magnitude as the calorigenic effect. Their finding suggests that tetrabromthyronine should be no more effective than thyroxine in suppressing the overactive gland of human Graves' disease. Nevertheless, as shown in Figure 5, a preliminary trial with this compound in a patient with hyperthyroidism, in a dosage of 5 to 20 mg. daily for twenty-nine days, caused a slight but not too definite drop in basal metabolism and a marked drop in the serum protein-bound iodine from 24γ to 8.8γ per cent on the twenty-first day of medication. Unfortunately, the blood iodine at the end of medication was not determined, but five days later, when the basal metabolism was rising, the blood iodine was only 11.7γ per cent. The results, in this case, are certainly suggestive of a relative diminution of the calorigenic effect and increase of the thyroid-depressing properties of tetra-

bromthyronine as compared with thyroxine but they require extensive confirmation. We hope to study the point further in the near future, not only with this compound, but also with tetrachlorthyronine and other analogues of thyroxine.

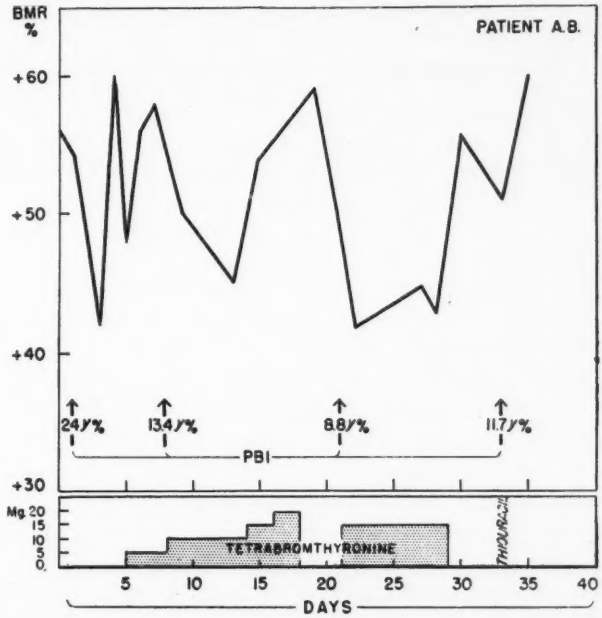

FIG. 5. The metabolic and serum protein-bound iodine (PBI) changes in a patient with hyperthyroidism receiving tetrabromthyronine. The initial dose of 5 mg. daily intravenously was gradually raised to 20 mg. daily. The drug was omitted on the eighteenth day and resumed on the twenty-first day for eight more days, in a daily dose of 15 mg. The values for serum PBI are indicated directly.

SUMMARY AND CONCLUSIONS

The bromine and chlorine analogues of thyroxine have been assayed in patients with spontaneous myxedema. Tetrabromthyronine is found to be one-seventeenth as active as thyroxine, and tetrachlorthyronine one two-hundred-and-fiftieth as active. This low order of calorigenic activity is desirable in a drug which could be used to depress the overactive thyroid of Graves' disease without at the same time increasing the excessive tissue metabolism. Although animal experiments indicate that tetrabromthyronine is not suitable in this respect, preliminary trial in one thyrotoxic patient does suggest that the thyroid-depressing property of tetrabromthyronine is greater than its calorigenic property.

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THYROID HORMONE-LIKE PROPERTIES OF TETRABROMTHYRONINE AND TETRACHLORTHYRONINE*†

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ALTHOUGH the important part played by iodine in the action of the thyroid hormone is thoroughly appreciated, its mode of action remains one of the intriguing mysteries of thyroid physiology. Baumann's (1896(1)) discovery of iodine in the thyroid gland, Kendall's (1915(2)) isolation of a substance from the thyroid containing 60 per cent iodine which was capable of abolishing all manifestations of thyroid insufficiency, and the synthesis of crystalline thyroxine by Harington and Barger (1927(3)) are significant landmarks in our increasing knowledge concerning the importance of iodine in thyroid physiology. Harington and Barger concluded from their studies that the physiologic activity of the hormonal compound is dependent upon its content of iodine.

Abderhalden and Wertheimer (1928(4)) tested the potency of certain analogs of thyroxine by comparing the effects of these compounds with those of thyroxine on the metamorphosis of tadpoles and axolotls. They found that tetrabromthyronine, a compound in which the iodine atoms of thyroxine are replaced with bromine atoms, had slight activity. They concluded that the molecular structure was an important feature of physiologic activity.

Gaddum (1930(5)) found tetrabromthyronine to have a calorigenic effect when given to rats in doses of 50 milligrams per kilogram of body weight. This response compared favorably to the response he obtained by injecting 2 to 4 milligrams of thyroxine per kilogram of body weight.

More recently Lerman (1948(6)) has studied the effect of tetrabromthyronine in myxedematous patients. He has observed that the administra-

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tion of tetrabromthyronine in daily doses of 15 to 20 milligrams results not only in an elevation of the basal metabolic rate to normal levels, but also in an alleviation of the clinical picture of myxedema.

With the above observations in mind, we undertook the studies herein reported with the following objectives: 1. To determine whether tetrabromthyronine[1] and tetrachlorthyronine[1] possessed any thyroid hormonal actions which could be compared to those of thyroxine. 2. To discover whether any thyroxine-like activity of the brominated and chlorinated thyronine molecules was the result of an *in vivo* iodination of the molecule. 3. To determine the effect of these agents on the production and/or release of thyroid-stimulating hormone by the anterior pituitary. 4. To compare the action of sodium bromide with that of potassium iodide on the development of iodine-deficient goiters.

METHODS

The method of Dempsey and Astwood (7) was used in evaluating the thyroxine-like activity of the various agents. These authors found that the minimum daily dose of thyroxine necessary to prevent the development of goiter in rats receiving thiouracil was 5.2 micrograms at 25°C. and 9.5 micrograms at 1°C. McGinty (8), who made observations on rats maintained at room temperature, found that the minimum daily dose of thyroxine given by subcutaneous injection necessary to prevent any iodine loss from the thyroid when animals were receiving thiouracil, was 20 to 30 micrograms.

In the four series described in Table 1, male rats of the Sprague Dawley strain weighing between 125 and 150 grams were maintained on a stock diet. Each group of a series contained 14 to 16 animals. Thiouracil was administered in the diet in a 0.2 per cent concentration except in series three, where it was given in the drinking water in an 0.1 per cent concentration. All series were maintained at room temperature except series three, which was maintained in the cold room at temperatures averaging 12°C. Subcutaneous injections of drugs were given daily in the dosages described subsequently. The experiments were of ten days' duration. On the tenth day the animals were heparinized and sacrificed by exsanguination under pentobarbital sodium anesthesia. Their thyroids were removed and weighed individually. These glands were pooled in lots of 4 to 5 and digested in 10 per cent potassium hydroxide for determination of total thyroid iodine. The method of Astwood and Bissell (9) was used in determining the thyroid iodine. The bloods from each group were pooled in two lots and

[1] These compounds were supplied by Professor C. R. Harington, Director of the National Institute for Medical Research, London, England.

TABLE 1

No. of rats	Thiouracil	*d-l* Thyroxine	Tetrabrom-thyronine	Tetrachlor-thyronine
		Series One		
15				
15	0.2% in diet			
15	0.2% in diet	5γ daily		
15	0.2% in diet	20γ daily		
15	0.2% in diet		75γ daily	
15	0.2% in diet		300γ daily	
		Series Two		
15				
16		20γ daily		
16			1000γ daily	
16	0.2% in diet			
14	0.2% in diet	20γ daily		
15	0.2% in diet		1000γ daily	
		Series Three		
13				
15		20γ daily		
16			1000γ daily	
16	0.1% in drinking water			
16	0.1% in drinking water	20γ daily		
16	0.1% in drinking water		1000γ daily	
		Series Four		
15				
15	0.2% in diet			
15	0.2% in diet	20γ daily		
15	0.2% in diet			2000γ daily
14	0.2% in diet			4000γ daily

Rats in series three were maintained at temperatures averaging 12°C. All other rats were maintained at room temperature. The experiments were all of ten days' duration.

their serum precipitable iodine was determined by the method of Man, Smirow, Gildea and Peters (10). The pituitaries were removed, weighed, pooled, and frozen, in which state they remained until their injection into chicks for assay of thyrotropic hormone.

Series one was divided into six groups. Group one received no treatment. The remaining five groups received thiouracil. The third and fourth

TABLE 2

No. of rats	Thiouracil in drinking water	Potassium iodide	Sodium bromide
		Series One	
9			
9		100γ I daily	
9			1500γ Br daily
9	0.1%		
8	0.1%	100γ I daily	
9	0.1%		1500γ Br daily
		Series Two	
12			
13		100γ I daily	
13			1500γ Br daily
13	0.1%		
13	0.1%	100γ I daily	
14	0.1%		1500γ Br daily
		Series Three	
14			
13		100γ I daily	
13			1500γ Br daily
13	0.1%		
11	0.1%	100γ I daily	
13	0.1%		1500γ Br daily
		Series Four	
14			
13		100γ I daily	
14			1500γ Br daily
14	0.1%		
15	0.1%	100γ I daily	
15	0.1%		1500γ Br daily

All rats were on the Remington diet throughout the experiments. Series one, three, and four were on the diet for twenty-five days. Series two was on the diet thirty-five days. Series one, two, and four received the experimental drugs the last ten days of the experiment. Series three received the experimental drugs beginning the first day and continuing for the duration of the experiment.

groups received thyroxine, 5 micrograms and 20 micrograms respectively; and groups five and six received tetrabromthyronine, 75 micrograms and 300 micrograms respectively.

Series two and three were each divided into six groups. The last three groups of each series received thiouracil. Groups two and five received

20 micrograms of thyroxine and groups three and six received 1 milligram of tetrabromthyronine.

Series four was divided into five groups. The last four groups received thiouracil. Group three received 20 micrograms of thyroxine and groups four and five received 2 and 4 milligrams respectively of tetrachlorthyronine.

Pituitaries were assayed for their thyrotropic activity in the following manner: the glands were homogenized and suspended in a saline solution. From a total of 22 to 24 milligrams (in the case of pituitaries from rats of series two and three) and of 10 milligrams (in the case of pituitaries from rats of series four) an equal aliquot was injected into three-day-old chicks on each of three days. On the fourth day the thyroids were removed, placed in 10 per cent neutral formalin, prepared in paraffin blocks, and sectioned at ten micra. Their mean acinar cell heights were then determined by the method described by Rawson and Salter (11).

In the four series described in Table 2, male rats of the Wistar strain weighing between 120 and 140 grams were maintained on an iodine deficient diet (Levine, Remington, and von Kolnitz, (12)). Each series was divided into six groups. Each group consisted of from 10 to 15 animals. The last three groups received thiouracil administered in a 0.1 per cent solution in the drinking water, beginning the day of the first injection. Groups two and five received 100 micrograms of iodine as potassium iodide and groups three and six received 1,500 micrograms of bromine as sodium bromide. Injections of these drugs were given daily in the stated dosages. On the last day of each experiment the animals were sacrificed by ether anesthesia, their thyroids removed and weighed individually.

Series one and four were on the deficient diet for twenty-five days and received the experimental drugs during the last ten days. Series two was on the deficient diet for thirty-five days and received the drugs during the last ten days. Series three was on the diet for twenty-five days and received the drugs throughout the experiment.

RESULTS

In Table 3 are recorded the effects of thyroxine, tetrabromthyronine and tetrachlorthyronine on thyroid weight, thyroid iodine, and serum precipitable iodine of rats treated with thiouracil. In series two and three the effects of these compounds were also studied in rats not given thiouracil. Statistically significant differences between groups are quoted if that difference is greater than three times the standard errors of the difference. As has been previously demonstrated, thyroxine in dosages of 5 and 20 micrograms daily was observed to prevent the development of goiters in rats receiving thiouracil at room temperatures. In series three, the ex-

TABLE 3

Treatment	Mean thyroid wt. mg./100 Gm. body wt.	Av. thyroid iodine, gamma/100 mg.	Serum precipitable iodine, gamma per cent
	Series One		
None	6.13 ±0.27	29.2	1.9
Thiouracil	17.7 ±2.03	0.9	1.0
Thiouracil +5γ thyroxine	5.98 ±0.23	35.8	2.6
Thiouracil +20γ thyroxine	6.72 ±0.39	29.0	5.2
Thiouracil +75γ tetrabromthyronine	13.2 ±1.43	0.97	1.2
Thiouracil +300γ tetrabromthyronine	6.35 ±0.23	8.25	1.2
	Series Two		
None	5.6 ±0.25	100.6	3.3
Thyroxine, 20γ	5.3 ±0.27	106.0	7.0
Tetrabromthyronine, 1 mg.	5.0 ±0.25	53.7	2.6
Thiouracil	11.5 ±0.54	7.6	1.1
Thiouracil +20γ thyroxine	5.7 ±0.23	46.0	29.1
Thiouracil +1 mg. tetrabromthyronine	6.6 ±0.20	16.2	15.8
	Series Three		
None	6.8 ±0.35	41.3	3.0
Thyroxine, 20γ	5.5 ±0.30	67.9	3.4
Tetrabromthyronine, 1 mg.	6.6 ±0.16	41.1	2.2
Thiouracil	19.7 ±1.28	4.6	1.6
Thiouracil +20γ thyroxine	7.2 ±0.39	28.9	6.7
Thiouracil +1 mg. tetrabromthyronine	11.9 ±0.65	10.2	2.9
	Series Four		
None	6.6 ±0.26	98.9	2.8
Thiouracil	14.5 ±0.64	2.9	10.2
Thiouracil +20γ thyroxine	5.1 ±0.20	64.6	7.3
Thiouracil +2 mg. tetrachlorthyronine	11.4 ±0.41	8.2	0.9
Thiouracil +4 mg. tetrachlorthyronine	10.6 ±0.40	17.0	1.4

Each group of a series contained 14 to 16 animals. The thiouracil was administered in the diet in a 0.2 per cent concentration except in series three, where it was given in the drinking water in a 0.1 per cent concentration. Thyronine, tetrabromthyronine, and tetrachlorthyronine were injected daily in the dosage given for the period of the experi-

periment conducted in the cold, 20 micrograms did not lower the thyroid weights of the thiouracil treated animals to the degree that thyroxine alone did. This difference is significant. Control thyroid weights in this series do not differ significantly from either of the two groups treated with thyroxine.

In the first series, loss of thyroid iodine in thiouracil-treated rats was prevented by daily doses of 5 and 20 micrograms of thyroxine. In series two and three where 20 micrograms was given, this finding was not repeated. There was inhibition of thyroid iodine loss, but not complete prevention.

With one exception the serum precipitable iodine of animals receiving thyroxine was distinctly above that of the normal controls. The animals maintained in the cold room, receiving only thyroxine, had values which appeared to be in the normal range. It should be noted that animals maintained in the cold room and receiving thyroxine had a significantly lower serum precipitable iodine than did those receiving thyroxine at room temperature. Also to be noted in series two and three is that rats treated with thyroxine and thiouracil had a higher precipitable iodine than did those rats which received thyroxine only.

Tetrabromthyronine was observed to inhibit the development of goiter in rats receiving thiouracil. Although a daily dose of 75 micrograms of tetrabromthryronine did not completely prevent the development of goiter, a daily dose of 300 micrograms was fully effective in preventing the goitrogenic effect of thiouracil. In series one and two, the thyroid weights of rats given thiouracil plus 300 or 1,000 micrograms of tetrabromthyronine did not differ significantly from those of untreated controls. In series two, tetrabromthyronine was also given without added thiouracil, and in these animals the thyroid weights are significantly lower than those of animals treated with the same amount of the drug and added thiouracil.

Loss of thyroid iodine in rats treated with thiouracil was not completely prevented by the addition of tetrabromthyronine. The loss does seem to be somewhat inhibited, as these figures for thyroid iodine are slightly higher than the values of thyroid iodine in animals receiving thiouracil only. Twice the amount of iodine is found in the thyroids from animals

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ment (ten days). All series were maintained at room temperatures (25°C.) except series three, in which the experiment was conducted in the cold room with temperatures averaging 12° C. Mean thyroid weights are given with their standard errors.

$$SE = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N(N-1)}}.$$

which received 1 milligram of tetrabromthyronine and thiouracil in series two, when compared with those values of thyroid iodine of animals receiving 300 micrograms of tetrabromthyronine and thiouracil in series one. However, if one calculates the percentage iodine remaining in the thyroids after the above treatment, based on 100 per cent in the control glands of each series, it will be seen that the thyroids of the animals receiving the greater amount of the drug have 16 per cent, whereas those receiving the smaller amount have 28 per cent of their iodine remaining.

In only one case did the serum precipitable iodine of animals treated with tetrabromthyronine rise above normal values. In series two, the group treated with thiouracil and 1,000 micrograms of tetrabromthyronine had an unreasonably elevated serum precipitable iodine, possibly due to inadvertent contamination before or during analysis. The serum precipitable iodine in animals receiving thiouracil and tetrabromthyronine in daily doses of 75 and 300 micrograms approached those values observed in rats receiving thiouracil only. The normal serum precipitable iodine in those rats treated with one milligram of tetrabromthyronine and thiouracil and maintained at the cold temperature should be noted. Those groups which received tetrabromthyronine without added thiouracil have values of serum precipitable iodine lying between control values and values found in the thiouracil treated animals.

Tetrachlorthyronine in daily doses of 2 and 4 milligrams did significantly inhibit the development of goiters in rats receiving thiouracil, but did not completely prevent it. It will be noted that there is marked iodine loss in the thyroids of these animals. However, the larger dose of tetrachlorthyronine seems to have partially inhibited the loss of thyroid iodine when compared with the figures obtained in the thiouracil-treated glands.

The serum precipitable iodine in rats receiving thiouracil and tetrachlorthyronine approached those levels usually found in animals treated with thiouracil. The high serum precipitable iodine found in this series for animals on thiouracil is completely out of keeping with other figures which we have obtained for animals treated with thiouracil alone, and suggests contamination with extraneous iodine.

The action of sodium bromide as compared with that of potassium iodide on the thyroids of rats on an iodine deficient diet is shown in Table 4. Potassium iodide containing 100 micrograms of iodine given daily was not only capable of inhibiting the iodine deficient goiter, but also of partially inhibiting the goitrogenic action of thiouracil.

A daily dose of sodium bromide containing 1,500 micrograms of bromine did not inhibit the goiter of the iodine deficient diet. In series one of Table 4 it will be seen that the thyroids of the controls had an average weight of 9.0 milligrams per 100 grams of body weight. The thyroids of animals

on the diet and receiving the daily injections of sodium bromide had an average weight of 7.1 milligrams per 100 grams of body weight. In no other experiment have we been able to get as great a deviation, although we proceeded in one instance in exactly the same manner (Table 4, series four).

At no time did the sodium bromide inhibit the goiter of animals treated with the deficient diet and thiouracil. In one instance (Table 2, series

TABLE 4

Treatment	Mean thyroid weights (mg. per 100 Gm. of body weight)			
	Series One	Series Two	Series Three	Series Four
None	9.0 ±0.42	10.1 ±0.30	13.9 ±0.71	9.9 ±0.69
100γ I	6.4 ±0.55	6.3 ±0.32	9.4 ±0.45	7.1 ±0.33
1500γ Br	7.1 ±0.60	9.7 ±0.37	14.6 ±0.54	11.3 ±0.73
Thiouracil	26.4 ±1.51	19.5 ±0.91	36.4 ±2.18	24.7 ±1.22
Thiouracil +100γ I	16.2 ±1.80	12.2 ±0.39	27.3 ±3.53	17.1 ±1.06
Thiouracil +1500γ Br	26.2 ±1.15	22.2 ±0.68	44.5 ±2.51	24.7 ±0.87

The rats in all series were on an iodine deficient diet (Levine, Remington, and von Kolnitz (12)), series one, three and four for twenty-five days, and series two for thirty-five days. Each group of a series contained 10 to 15 animals. The thiouracil was administered in the drinking water in a 0.1 per cent concentration, starting the day of the first injection and continuing throughout the experiment. I and Br were injected daily as potassium iodide and sodium bromide respectively, in the dosages shown. Injections were given the last ten days of the experiment in series one, two, and four, and throughout the twenty-five days in series three. Mean thyroid weights are given with their standard errors.

$$SE = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N(N-1)}}.$$

three) where the injections were given over the longest time, twenty-five days as against ten days in the other series, the goiters of animals receiving sodium bromide and thiouracil were significantly larger than those of rats treated with thiouracil only.

The results of the pituitary assays are recorded in Table 5. Whereas significant thyrotropic activity was demonstrated in the pituitaries of rats which had received no treatment, no thyrotropic activity was demonstrated in pituitaries taken from rats treated with thiouracil. All pituitaries from animals treated with thyroxine and tetrabromthryonine and with these agents in conjunction with thiouracil, showed varying but significant

amounts of thyrotropic activity. There was an increase of 1 to 2 micra in the mean cell heights in the assays of pituitaries from the tetrabromthyronine-treated animals above those in the assay of the pituitaries from the thyroxine-treated animals. Only in the second group of series three, in

TABLE 5

No. of chicks	Pooled pituitary source: and treatment rats received	Average mean acinar cell height (micra)
15	(Chick controls)	3.4
	From Series Two, Table 3	
4	None	6.5
4	Thyroxine, 20γ	6.6
4	Tetrabromthyronine, 1 mg.	8.1
4	Thiouracil	3.4
3	Thiouracil +20γ thyroxine	6.1
4	Thiouracil +1 mg. tetrabromthyronine	7.2
	From Series Three, Table 3	
3	None	8.1
3	Thyroxine, 20γ	7.9
3	Tetrabromthyronine, 1 mg.	8.0
3	Thiouracil	4.4
3	Thiouracil +20γ thyroxine	6.4
3	Thiouracil +1 mg. tetrabromthyronine	8.4
	From Series Four, Table 3	
6	None	5.9
6	Thiouracil	3.0
5	Thiouracil +20γ thyroxine	4.1
4	Thiouracil +2 mg. tetrachlorthyronine	3.9
4	Thiouracil +4 mg. tetrachlorthyronine	3.9

Thyrotropic hormone assays of pituitaries from series two, three, and four of Table 1. In series two and three, from 22 to 24 mg. of pituitary tissue was injected, and in series four, 10 mg. of pituitary tissue was injected in each chick, an equal aliquot on each of these days.

which thyroxine alone was used, did the mean acinar cell height approach those of the tetrabromthyronine-treated animals.

Assays of pituitaries removed from the tetrachlorthyronine-treated rats revealed slight but significant thyrotropic activity. However, the thyrotropic activity in these pituitaries was less than that demonstrated in the pituitaries of the untreated rats. These differences might have been more

TABLE 6. SUMMARY OF RESULTS

Figures represent the per cent change from untreated controls

(taken from Table 3)

Series	Treatment	Room temp., (° C.)	Mean thyroid weight	Mean thyroid iodine	Serum precipitable iodine	T.S.H. in pituitary
1	None		100%	100%	100%	Normal
2						
3						
4						
2	20γ Thyroxine	25°	95%	105%	*212%*	Normal
3		12°	81%	164%	113%	Normal
2	1,000γ Tetrabromthyronine	25°	89%	*53%*	79%	Increased
3	(T Br T)	12°	97%	100%	73%	Normal
1		25°	*288%*	*3%*	*53%*	
2	Thiouracil (TU)	25°	*215%*	*8%*	*33%*	*Reduced*
4	alone	25°	*220%*	*3%*	(364%)	*Reduced*
3		12°	*290%*	*11%*	*53%*	*Reduced*
1	TU+Thyrox. 5γ	25°	98%	123%	137%	
1	TU+Thyrox. 20γ	25°	110%	99%	*274%*	
2	TU+Thyrox. 20γ	25°	102%	*46%*	*882%*	Normal
4	TU+Thyrox. 20γ	25°	77%	65%	*260%*	Reduced
3	TU+Thyrox. 20γ	12°	106%	70%	*223%*	Reduced
1	TU+TBrT 75γ	25°	*215%*	*3%*	*63%*	
1	TU+TBrT 300γ	25°	104%	*28%*	*63%*	
2	TU+TBrT 1,000γ	25°	118%	*16%*	(479%)	Normal
3	TU+TBrT 1,000γ	12°	*175%*	*25%*	97%	Normal
4	TU+TClT 2,000γ	25°	*173%*	*8%*	*32%*	Reduced
4	TU+TClT 4,000γ	25°	*161%*	*17%*	*50%*	Reduced

Those figures which are in italics show significant deviations from the control figures. The two values for serum precipitable iodine which have been enclosed in parenthesis seem unreasonably high. This may have been due to contamination of the blood sample with extraneous iodine.

striking had the same amount of pituitary tissue been used in these assays as in those above. A summary of all the results based on percentage change from untreated controls is presented in Table 6.

DISCUSSION

We have observed a thyroid hormone-like action of tetrabromthyronine and tetrachlorthyronine which appears to follow the pattern now accepted for thyroxine. One wonders if an increased dosage of the tested compounds might clarify the question of the prevention of iodine loss from the thyroids. Just how physiologic are the doses that have been given, we do not know. No toxic effects were encountered with the dosages used, but they might occur with higher doses.

One explanation for these observed actions of tetrabromthyronine and tetrachlorthyronine is that these compounds may be iodinated *in vivo* and thus may exert a thyroxine-like action. The fact that animals treated with both thiouracil and these agents were observed in some instances to have serum precipitable iodines which approached those found in animals treated with thiouracil alone, would negate this explanation. Notwithstanding these low iodine values, animals receiving the noniodinated analogs of thyroxine had partial to complete inhibition of goiter production.

It will be noted that there was wide variation in the mean thyroid iodine values of our control animals. Animals with mean values of 30 micrograms per 100 milligrams were sacrificed in May; those with 100 micrograms per 100 milligrams in August; those with 40 micrograms per 100 milligrams also in August, but were maintained at room temperatures of 12°C. Fenger (13) in a study done at the Armour laboratories concerning the thyroid iodine content of hog, sheep, and beef thyroid glands found a definite seasonal variation in these values. The peak was reached in August whereas lower values were found in the cooler months. Such a seasonal variation would explain the values we found in our control animals.

Since it has been demonstrated that thiouracil goiters will not develop in hypophysectomized animals, it seemed logical that inhibition of the goiter by the agents used might affect the thyrotropic hormone of the pituitary. The most logical point of action seemed the pituitary itself, although Cortell and Rawson (14) have observed that the administration of thyroxine to hypophysectomized rats depresses the response of the animal's own thyroid gland to injected thyrotropic hormone.

The presence of thyrotropic hormone in the pituitaries of animals receiving thyroxine or tetrabromthyronine in like amounts to that found in the pituitaries of the control rats suggests an inhibition of release rather than one of formation. One might postulate the emptying of the pituitaries of thyrotropic hormone in those animals receiving the thiouracil only.

Having observed that these two synthetic compounds with the same general molecular structure as thyroxine have a thyroxine-like action when injected *in vivo*, we began to consider the ability of the thyroid to utilize

bromine in the synthesis of a thyroid hormone. Baumann, Sprinson, and Marine (15) suggested that the thyroid can distinguish only imperfectly between iodine and bromine. It may be possible, they state, that in the absence of iodine, bromine is selectively seized by the thyroid. Perlman, Morton, and Chaikoff (16), using radioactive bromine, calculated the values for the extracellular fluid space of tissues according to the formula of Hastings and Eichelberger, and obtained values for the extracellular volume of the thyroid greater than the total weight of the gland. They concluded that the thyroid showed a greater uptake of the labeled bromine than any of the other tissues that they tested, and that this uptake could not be explained on the basis of simple diffusion from the serum.

We were unable to inhibit the development of iodine-deficient goiters with rather large amounts of bromine. Perhaps the diet was not sufficiently deficient in iodine, or the amount of bromine was so great as to be completely unphysiologic. Our experiments do suggest that the bromine could not be utilized by the thyroid to synthesize a hormone. Granted that large amounts of the brominated compound would be needed, it still seems logical that some inhibition of the goiter should occur if the gland is capable of the brominating process. However, we have been unable to show any such action. The injected iodine clearly inhibited the goiters formed in rats on the deficient diet.

SUMMARY

Tetrabromthyronine and tetrachlorthyronine have been compared with thyroxine by determining their effects on the development of goiter, on the loss of thyroid iodine, on the serum precipitable iodine, and on the pituitary's thyrotropic activity, in rats receiving thiouracil.

It has been observed that thyroxine in daily doses of 5 and 20 micrograms prevents the development of goiters in rats receiving thiouracil at room temperature, and that 20 micrograms daily prevents the development of goiter in animals maintained at temperatures of 12°C. Daily doses of 5 and 20 micrograms did prevent loss of thyroid iodine in rats receiving thiouracil in one experiment. Others showed only partial inhibition with 20 micrograms.

Tetrabromthyronine in daily doses of 300 micrograms or more did prevent the development of goiters in rats receiving thiouracil at room temperature. One milligram of tetrabromthyronine did not completely prevent the development of the goiter in rats receiving thiouracil at 12°C. In no instance did tetrabromthyronine completely inhibit the loss of thyroid iodine.

Tetrachlorthyronine in daily doses of 2 and 4 milligrams, administered

to rats receiving thiouracil, only partially inhibited the development of goiter; and inhibition of loss of thyroid iodine was minimal.

Although the development of goiters was partially to completely inhibited by the administration of tetrabromthyronine and tetrachlorthyronine in rats receiving thiouracil, the animals were observed to have normal or decreased levels of serum precipitable iodine.

The pituitaries of rats treated with thiouracil were found to possess no thyrotropic activity. Animals treated with thiouracil and thyroxine or with tetrabromthyronine and thiouracil were observed to possess like amounts of thyrotropic activity when compared with the thyrotropic content of the pituitaries of untreated rats. The pituitaries of rats treated with thiouracil and tetrachlorthyronine contained measurable amounts of thyrotropic hormone, but less than the controls.

Sodium bromide did not inhibit the iodine-deficient goiter. Potassium iodide did inhibit this goiter and did also partially inhibit the goiter produced by the addition of thiouracil to the iodine-deficient diet.

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RADIOIODINE THERAPY OF METASTASES FROM CARCINOMA OF THE THYROID: A SIX-YEAR PROGRESS REPORT*§

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IN the past six years, 30 patients with metastases from carcinoma of the thyroid have been studied by our group at Montefiore Hospital. Of these, 12 were treated with radioactive iodine. On admission every patient was given a tracer dose of radioiodine and checked with a Geiger counter for uptake in the metastases. However, our cumulative experience shows that in the presence of normal non-neoplastic thyroid tissue the metastases do not usually show uptake. For patients with a proven diagnosis of metastatic carcinoma of the thyroid we now regard radiation or surgical thyroidectomy as a basic first step in treatment. In this manner, uptake can be induced in the metastases of most patients. After uptake has been demonstrated, the patient is treated with multiple large doses—usually 100 millicuries or more—of radioactive iodine (I^{131}).

In June 1947 we reported the first successful induction of uptake of radioiodine in metastases from thyroid carcinoma. This was accomplished by thyroidectomy (surgical or radiation) or by injection of exogenous thyroid stimulating hormone (T.S.H.) (1, 2). Thyroidectomy increases the T.S.H. production in the pituitary and removes the competition of the thyroid gland for both the T.S.H. and the iodine, thus enabling the tumor tissue to take over the function of the normal gland to varying degrees. This effect of thyroidectomy on the function of metastatic thyroid carcinoma was later confirmed by Rawson *et al.* (3).

Although the degree of uptake of radioiodine by the thyroid tumors is correlated in a general way with the degree of histologic differentiation, this is not always true. For example, we have several cases of well differentiated thyroid tumors which did not take up any radioiodine until total

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thyroidectomies had been performed. On the other hand, we have sections obtained from other patients which show radioiodine concentration in nondifferentiated areas and no concentration in some of the highly differentiated areas within the same section.[1]

Since 1943, we have treated a total of 12 patients with radioactive iodine (Table 1). Seven of this group are still living—3 of them greatly improved;

TABLE 1. EVALUATION OF EFFECT OF RADIOIODINE (I*) THERAPY
(MAY 24, 1949)

Patient	Period of I* therapy	Total I* administered I^{131} (millicuries)	Clinical result
B.B. ♂56	6 years (3/11/43–present)	111† 397	Greatly improved.
A.B. ♀67	5 years (5/5/44–present)	934	Stationary.
M.S. ♀59	3½ years (July '44–Jan. '48)	268† 686	Improved. Died later.
D.Y. ♀69	6 months (6/5/46–1/3/47)	202	No improvement. Died.
L.O. ♀41	2 months (10/17/46–12/25/46)	173	No improvement. Died.
J.F. ♂60	2¼ years (1/6/47–present)	665	Greatly improved.
L.L. ♀56	2 years (Jan. '47–Nov. '48)	539	Improved. Died later.
S.W. ♀40	1½ years (10/21/47–present)	694	Greatly improved.
L.G. ♂56	1 year (12/2/47–late '48)	194	Improved. Died later.
D.S. ♀20	1½ years (12/8/47–present)	161	Improved.
F.L. ♀49	9 months (8/3/48–present)	484	Stationary.
B.L. ♀60	5 months (Dec. '48–present)	195	Improved.
TOTALS: 12	2 months – 6 years	200–1,000 per patient	*7 Living:* 3 greatly improved 2 improved 2 stationary *5 Dead:* 3 initially improved 2 unimproved

† I^{130}.

[1] Representative histologic sections are shown in Figures 5 and 9, *J. Clin. Endocrinol.* **8:** 423–432 (June) 1948.

FIG. 1. Patient B.B. Photomicrograph of metastatic tumor in the twelfth thoracic vertebra (×400). Note follicular architecture and traces of granular colloid-like material (c) in some follicles. November, 1939.

2 moderately improved; and 2 in a stationary condition. Of the 5 who died, 2 (D.Y. and L.O.) were obviously moribund when they came under our care, and showed no improvement. However, the remaining 3 (M.S., L.G. and L.L.) showed sustained improvement and their lives were unquestionably extended by the radioiodine therapy. L.L. died with jaundice and fever at another hospital. We suspect that she succumbed to infectious hepatitis rather than to the carcinoma, but no details are available.

The radiation delivered to the tumor is not directly proportional to the quantity of I^{131} (millicuries) used. It depends rather upon the concentration of radioiodine in a given tumor as well as on the distribution of the isotope within that tumor. We have observed no definite correlation between the number of millicuries administered and the clinical results. We noted however, that if the radioiodine is going to be effective, improvement becomes apparent in the early stages of treatment.

We shall not go into detail regarding all the patients listed in the preceding table, but shall limit ourselves to a brief discussion of our oldest patient (B.B.), on whom preliminary reports were made in 1945 (4) and 1946 (5).

This patient came to our attention at Montefiore Hospital six years ago, presenting a picture of Graves' disease. His thyroid had been completely removed eighteen years before and the histologic diagnosis was adenocarcinoma of the thyroid. Two years prior to admission to our hospital, a tumor in his back—believed to be a lipoma—was excised and proved to be thyroid adenocarcinoma. A photomicrograph of this tissue (Fig. 1) shows that we are dealing here with a highly cellular, well differentiated tumor containing follicles with colloid.

Immediately following the removal of the tumor, the patient had a thyroid crisis and, in the subsequent two years, developed multiple metastases in the lungs, skull, rib, spine, pelvis and femur.

Shortly after admission to our hospital, radioiodine uptake was demonstrated in all the lesions. Subsequent study of uptake patterns in the various metastases showed consistent similarity and, therefore, readings over a given lesion were considered representative of the behavior of the others.

After some preliminary therapy, a biopsy specimen was removed from the rib metastasis (Fig. 2). Histologically, this tissue was similar to that previously obtained from the back, showing highly differentiated adenocarcinoma of the thyroid with alveoli and colloid. Some necrotic areas were present, which at that time were considered by Dr. Marine to be "consistent with radiation damage." From this biopsy material the first successful radioautograph of a metastasis from thyroid carcinoma was ob-

FIG. 2. Patient B.B. Photomicrograph of metastatic tumor in second rib (×400). Note follicular structure and colloid-like material (c) in a few follicles. November 16, 1943.

tained (5). It showed radioiodine distribution throughout the viable tumor tissue.

Radioiodine therapy was continued and the patient's clinical picture showed striking improvement. The pain in the lesions disappeared and the hyperthyroid state was gradually transformed into one of hypothyroidism. The patient then developed myxedema and is now receiving desiccated thyroid therapy.

Figure 3 shows the change in his appearance during this period as a result

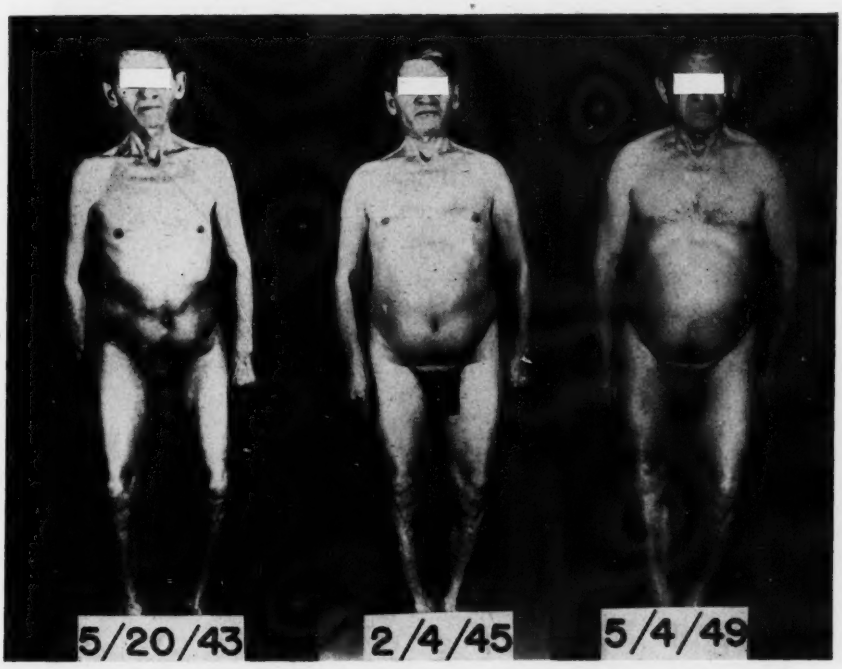


FIG. 3. Patient B.B. Change in appearance from hyperthyroidism to hypothyroidism as a result of radioiodine therapy.

of radioiodine therapy. The patient who, six years ago, was bedridden and required opiates, is now moving about freely and has no pain. Roentgenologically, no new metastases have appeared, the pulmonary metastases have completely disappeared and the osseous shadows (lesions?) have shown neither increase nor decrease in size. External measurements with the Geiger counter, which initially showed high readings, have shown no uptake in the past year.

On May 4, 1949, two days after administration of a test dose of 10

millicuries of radioiodine, a cranioplasty was performed on our patient by Dr. S. W. Gross. A piece of skull was removed, including the site of a metastasis. Figure 4 is a photograph of the skull lesion *in vivo* at the time of operation. The glistening, whitish area corresponds to the lesion. The same

FIG. 4. Patient B.B. Skull lesion *in vivo* at time of operation, May 4, 1949 (external aspect). The glistening whitish area is produced by highlights over the region of the metastasis.

portion of the skull, *ex vivo*, is shown in Figure 5. The region corresponding to the lesion is shown in the irregular, darkened area, measuring about 2.3×1.5 centimeters. The tumor area is outlined more clearly when viewed from the dural aspect (Fig. 6). On the inner aspect of the skull one sees a sharply circumscribed, purplish mass measuring 3.2×2.3 centimeters, pro-

truding somewhat above the surface, with many hemorrhagic areas. It is soft in consistency and abuts against the ragged edges of the necrotic skull. The purplish soft tumor mass shown in Figure 6 corresponds to an area of rarefaction in the roentgenogram of the removed section of the skull (Fig. 7).

A section of decalcified bone in the region of the metastasis with some adjacent soft tumor tissue is shown in Figure 8. Alongside the eosin-

FIG. 5. Skull lesion *ex vivo* immediately after operation (external aspect).

hematoxylin section is its radioautograph, showing scattered areas of radioiodine concentration, which is especially marked in the region of the soft tumor mass. This section is completely devoid of viable tumor tissue and the areas of radioiodine concentration correspond to regions of necrotic material. Figures 9A and 9B show two eosin-hematoxylin stained sections of the soft tumor mass with their corresponding radioautographs. These

FIG. 6. Same lesion from dural aspect.

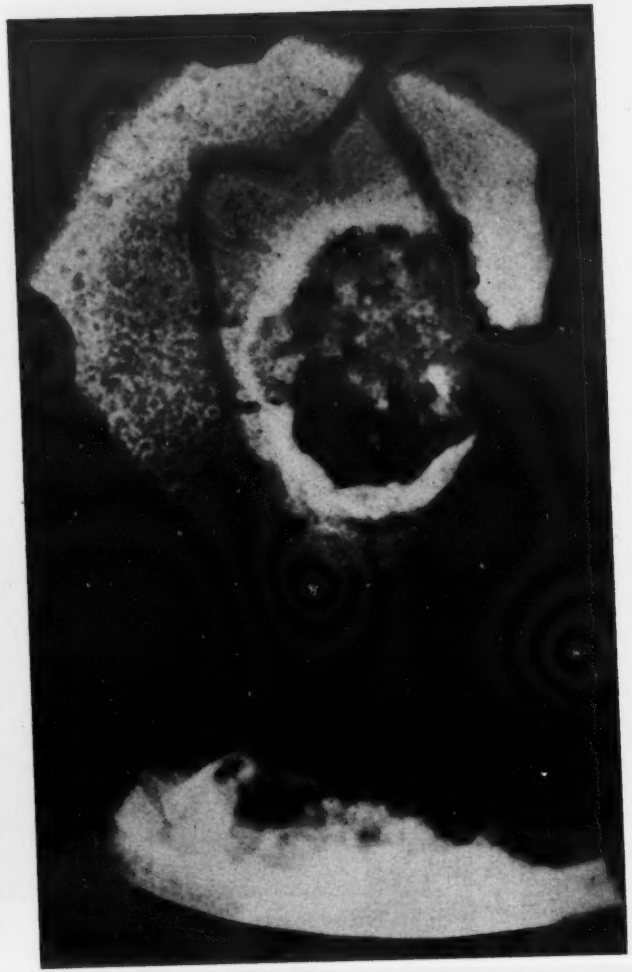
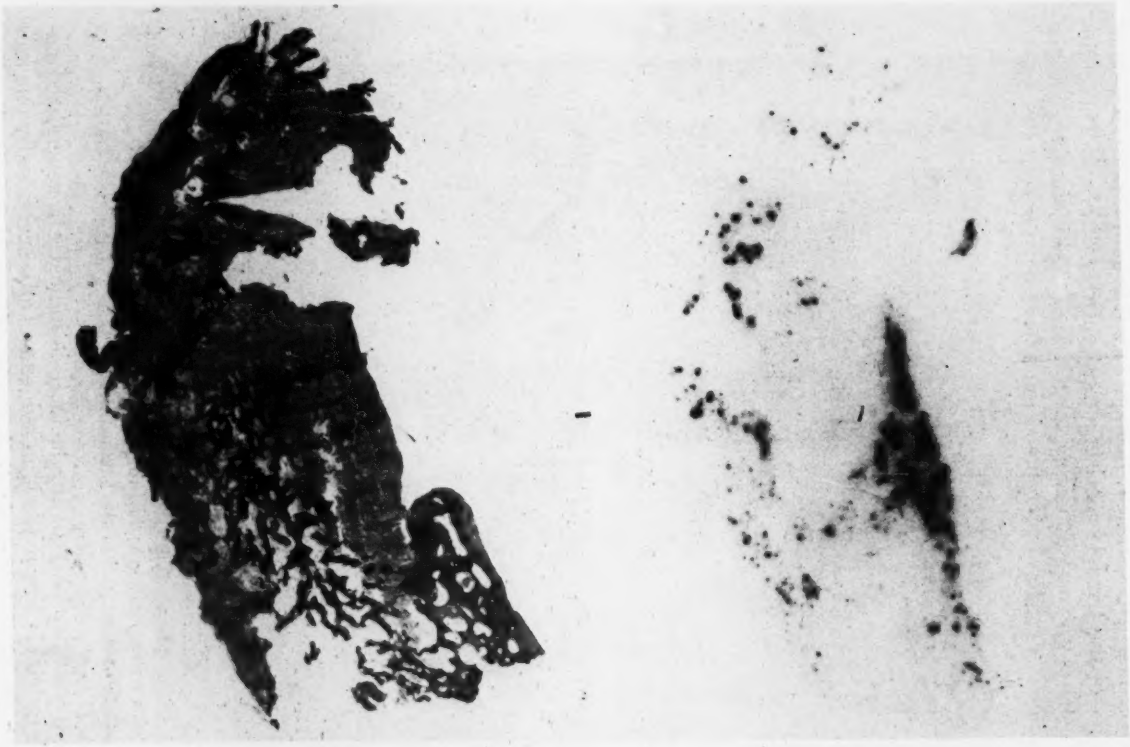


FIG. 7. Roentgenogram of excised piece of skull—direct and lateral views.

FIG. 6. Same lesion from dural aspect.

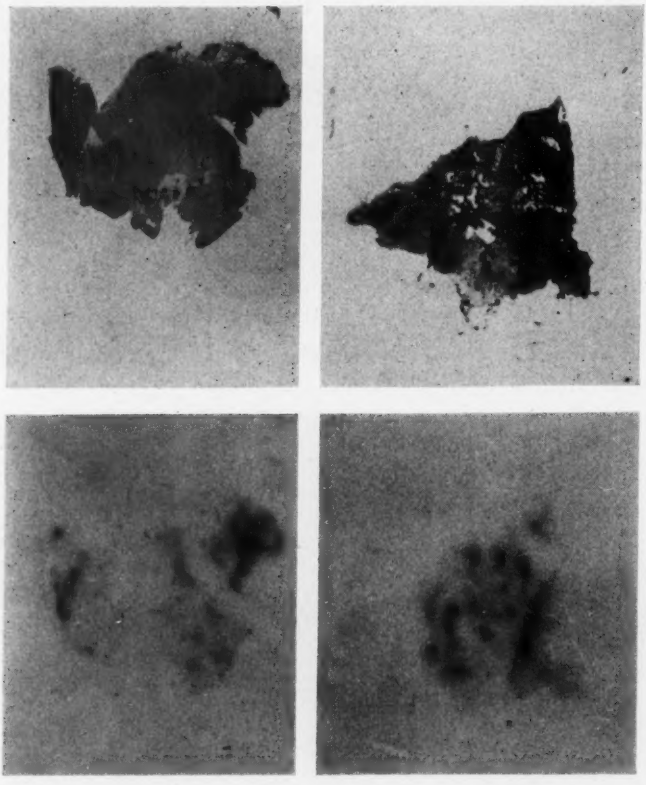
FIG. 7. Roentgenogram of excised piece of skull—direct and lateral views.



A B

FIG. 8. Decalcified section of skull metastasis
A. Eosin-hematoxylin stain (×9).
B. Radioautograph

sections demonstrate that radioiodine has localized in areas where no viable tumor cells can be demonstrated. In Figure 9B it is of interest to note that the left apex of the triangle consists almost entirely of blood. The radioautograph shows no corresponding dark shadow, thus indicating that the radioiodine concentration in the blood must have been very low.



A B

FIG. 9. A. Eosin-hematoxylin stained section from necrotic tumor mass of skull metastasis, with corresponding radioautograph (×9).

B. Same, from another part of the tumor mass.

Further histologic study of the metastasis indicates a generalized necrosis with no viable tumor tissue in evidence. Only occasionally one sees ghosts of deceased tumor cells, singly or in groups. Here and there are scattered phagocytic cells, some of them containing blood pigment (Figs. 10A, B, C and D). In some areas one finds connective tissue proliferation. This is especially well shown in Masson-stained slides.

FIG. 10A. Eosin-hematoxylin stained section of part of soft tumor mass of skull metastasis (×170). There is a complete absence of viable tumor cells. Note the disappearance of the histologic patterns seen in Figures 1 and 2.

The surprising fact is that the necrotic debris from the destroyed tumor tissue can take up or concentrate radioiodine. We do not know the reason for this phenomenon, but have postulated that products of the breakdown of the thyroid tumor cells are capable of combining selectively with iodine.

FIG. 10B. Another section from soft tumor mass of skull metastasis (eos.-hem. ×170).

It is also possible that this reaction is similar to the one involved in the staining of amyloid by iodine solution, and is therefore independent of the thyroid tumor origin.

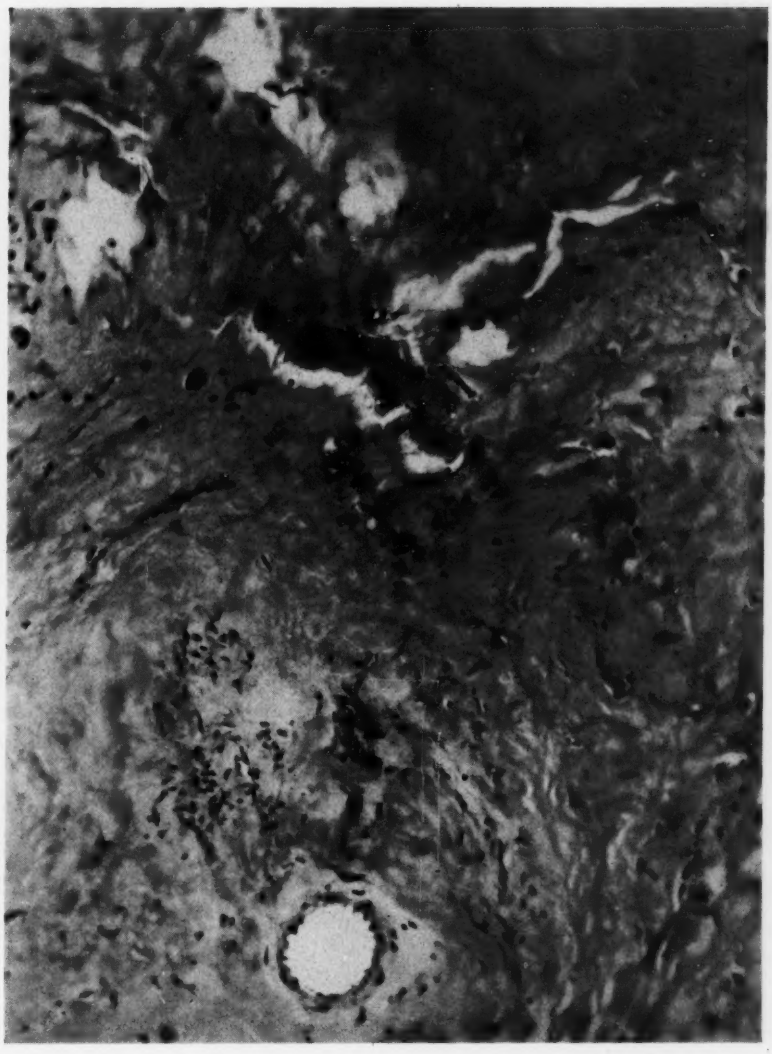


FIG. 10C. Another representative section from soft tumor mass of skull metastasis (eos.-hem. ×170).

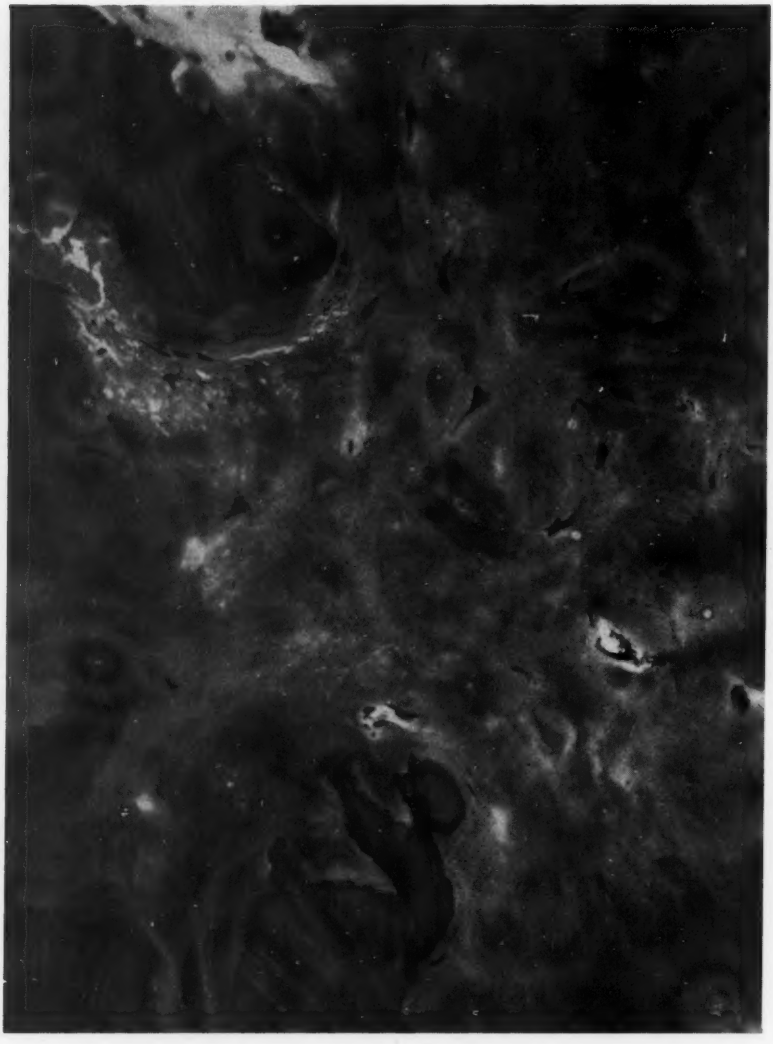


FIG. 10D. Eosin-hematoxylin stained section of decalcified bony part of skull metastasis (×170).

SUMMARY

1. In the past six years, we have studied 30 patients with metastatic carcinoma of the thyroid. Twelve of this group were treated with radioactive iodine. Seven are still living—3 of them greatly improved, 2 moderately improved, and 2 in a stationary condition. Of the 5 who are dead, 3 showed initial improvement and 2, who were admitted in terminal stages, no improvement whatever.

2. In cases in which metastases showed no initial radioiodine uptake, such uptake was induced by either thyroidectomy (surgical or radiation) or injections of thyrotropic hormone.

3. One of the treated patients was followed for six years after the initiation of therapy. A recently excised skull metastasis showed complete necrosis with no evidence of viable tumor tissue. It was found that the necrotic debris from the destroyed metastatic tumor could still concentrate radioiodine.

Acknowledgments

Since August, 1946, radioactive iodine used in this work has been obtained from the United States Atomic Energy Commission, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

The cooperation of Dr. Louis Leiter, Chief of the Medical Division, Dr. Daniel Laszlo, head of the Neoplastic Service, and Dr. Harry Zimmerman, Chief of the Laboratory Division, of Montefiore Hospital made this work possible.

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THE TREATMENT OF METASTATIC THYROID CANCER WITH RADIOACTIVE IODINE: CREDITS AND DEBITS*†

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THE first attempts to localize radioactive iodine in cancer of the thyroid were reported in 1940 by Hamilton, Soley, and Eichorn. Although they were unable to detect significant quantities of radioactive iodine in the 2 cancers of the thyroid that they studied, they wisely concluded that, "It is possible that in other cases of carcinoma of the thyroid, radioiodine may be stored in the affected tissues in quantities sufficient for treatment" (1).

The first successful attempt to demonstrate selective concentration of the isotope in thyroid cancer was reported in 1942 by Keston *et al.* (2). Therapeutic efforts were not undertaken however, because only one of the several metastases could be shown to collect iodine. A subsequent report in 1944 by the same authors (3) indicated that all metastases, except the one which concentrated iodine, were undifferentiated.

Seidlin, Marinelli and Oshry (4) in 1946 reported the first case in which not only selective collection of iodine by metastases of thyroid cancer was observed, but in which unmistakable improvement in the patient's over-all condition resulted from the administration of therapeutic quantities of I^{131}.

The following report is a summary of certain observations made at Memorial Hospital in patients having metastatic cancer of the thyroid who have been subjected to study and treatment with radioactive iodine. We propose to discuss not only the credits due this type of study and treatment but also the debits which have accrued. Under credits we shall discuss, in addition to the therapeutic effects of this form of treatment, the methods that we have used in altering the function of such cancers. We consider these studies as credits because of the information they have revealed regarding the physiology of thyroid cancer. On the debit side we propose to discuss the toxic effects observed in our patients subjected to intensive treatment with radioactive iodine.

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Clinical Material

These studies have been limited to 25 patients having proved cancer of the thyroid with metastases beyond the confines of the neck, *i.e.*, pulmonary and skeletal metastases. Of these 25 patients, only 1 had metastatic tumor tissue which concentrated enough radioactive iodine to justify administering this isotope in a therapeutic dose before subjecting the patient to removal of the normal thyroid or to other procedures which will be discussed.

Methods of altering function of the metastases

In this study of thyroid cancer, considerable effort has been directed toward stimulating metastatic thyroid cancer to assume the iodine-concentrating function of normal or of hyperplastic thyroid tissue. As we reported last year before the annual meeting of the American Goiter Association (5), complete surgical removal of the normal thyroid, or its destruction with radioactive iodine has been found to be a useful procedure in converting certain apparently nonfunctioning or poorly functioning metastatic cancers of the thyroid to functional states with the ability to collect iodine.

As of the present date, total removal or destruction of the normal thyroid in patients having metastatic carcinoma of the thyroid has been carried out in 23 patients.[1] One or more metastases in 12 of these patients have been observed to concentrate considerably more iodine than before removal of the normal thyroid. This is illustrated by the following case history:

M. A. (*M. H. #86407*), a 38-year-old white married housewife, was admitted to Memorial Hospital on June 6, 1947. She had had a hemithyroidectomy in 1943 for "goiter," but no slides from this original operation were available. Pain appeared in the back and right hip in May 1945 and became progressively worse, eventually combining with weakness of the lower extremities to confine the patient to bed. X-ray examination made in December 1945 revealed osteolytic lesions in the first and second lumbar vertebral bodies, the second and twelfth ribs, and the right ilium. A biopsy of the large lumbar spine lesion in April 1946 revealed a follicular adenocarcinoma of the thyroid.

A preliminary tracer dose of I^{131} given on June 14, 1947, showed a concentration of 3.2c/m/μc (at 15 cm.) in the thyroid[2] and only 0.32c/m/μc (skin contact) in the lumbar tumor. In an attempt to destroy the normal thyroid, I^{131} in a dose of 65.5 millicuries

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[1] The 21 patients reported in 1948 (5) included 8 cases studied at the Massachusetts General Hospital and 13 subjects studied at the Memorial Center for Cancer Research. The present report includes only those patients studied at the Memorial Center.

[2] "Counts" per minute per microcurie of administered dose. This expression is used in situations requiring that the Geiger-Müller tube be placed in contact with tissue overlying a tumor which is being measured for content of radioactivity. It is employed here only for the purpose of comparing relative activity at any one site as a function of time.

was given on July 16, 1947. Two months later, on September 15, another test dose was administered. This revealed a decrease to 0.18c/m/μc in the thyroid and an increase to 3.8c/m/μc in the tumor. This change permitted the subsequent administration of three therapeutic doses of 91 mc., 160 mc., and 200 mc. (Fig. 1).

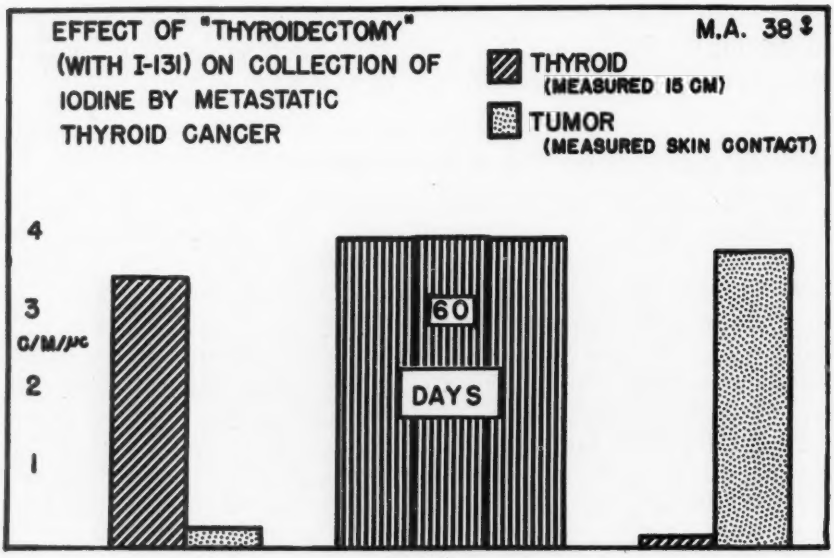

FIG. 1. *The effect of "thyroidectomy" on the collection of radioactive iodine by metastatic thyroid cancer. Mrs. M. A., M. H. #86407.*

The cross-hatched columns represent the "iodine pickup" by thyroid tissue in the neck. The stippled columns represent the "iodine pickup" by a metastasis in the spine. The activity is recorded as counts per minute divided by microcuries administered. Measurements over the thyroid were made at 15 cm. from the skin; those over metastasis were made at the skin surface. On the left are recorded measurements made before the normal thyroid was destroyed. Columns on the right show the observations made on the pickup of iodine administered sixty days after destroying the normal thyroid.

We have also studied the effect of thyroid-stimulating hormone and of prolonged treatment with thiouracil on the collection of radioactive iodine by metastatic cancer of the thyroid. In 3 out of 6 patients whose normal thyroid had been removed, it was observed that treatment with thyroid-stimulating hormone (30 mg.[3] daily for five or more days) resulted in an increased avidity for radioactive iodine. One patient having three skeletal metastases was treated with thyroid-stimulating hormone before his normal thyroid was removed. In this patient, the normal thyroid showed

[3] Assayed 3.8 Evans chick units per milligram. Supplied by Dr. John R. Mote, of the Armour Laboratories.

no significant increase in iodine-collecting capacity, whereas all three metastases were observed to collect three to four times as much radioactive iodine as they did before administration of thyrotropic hormone (Fig. 2).

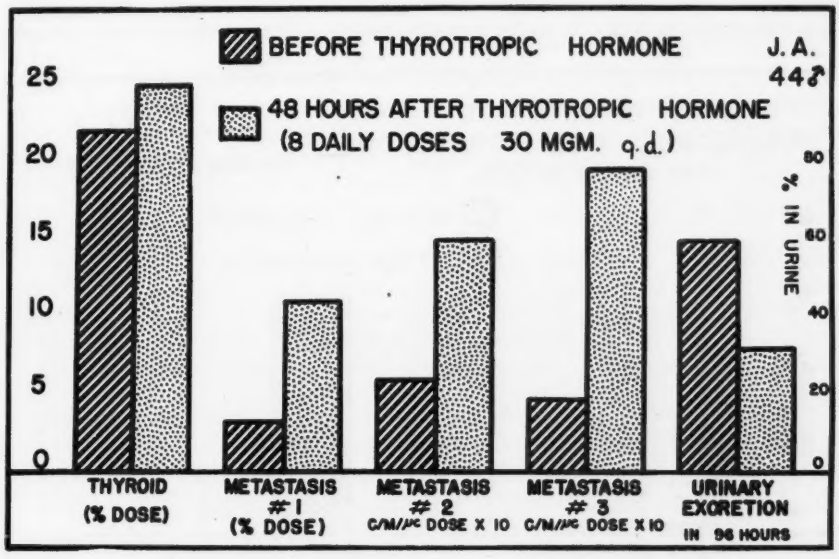

FIG. 2. *The effect of thyrotropic hormone on the pickup of radioactive iodine by metastatic cancer of the thyroid. Mr. J. A., M. H. #77311.*

The cross-hatched columns represent the "pickup of radioactive iodine" by the thyroid and 3 metastases, as well as the urinary excretion of radioactive iodine, prior to treatment with thyrotropic hormone. The stippled columns represent the per cent pickup by thyroid and metastases and the urinary excretion after stopping treatment with thyroid-stimulating hormone. It will be noted that the pickup on all 3 metastases was increased after such treatment whereas there was no significant change in the thyroid's avidity for radioactive iodine. The urinary excretion of radioiodine was decreased following treatment with thyroid-stimulating hormone.

Ten thyroidectomized patients whose metastatic lesions had acquired significant degrees of avidity for radioactive iodine have been treated for several weeks to months with thiouracil or propylthiouracil. After stopping administration of these drugs, a marked increase in the iodine-collecting capacity of the metastatic lesions has been observed in 7 patients (Fig. 3). The effects of either thyrotropic hormone or thiouracil are illustrated by the following case history (Figs. 2 and 3):

J. A. (M. H. #77311), a Roumanian Jewish businessman was admitted to Memorial Hospital on January 16, 1948, for study of a known metastatic carcinoma of the thyroid. He had first consulted a physician in November 1944 because of persistent pain in the

right hip. X-ray studies revealed an osteolytic lesion in the intertrochanteric region of the right femur. An aspiration biopsy done in April 1945 revealed a follicular adenocarcinoma of thyroid origin. Although an enlarged firm left lobe of the thyroid was discovered, the patient refused thyroidectomy. X-radiation was administered to both the thyroid and the femoral metastasis (4,200 r to each lesion). Considerable regression was observed in both lesions. Relief of pain in the right hip was also obtained. In April

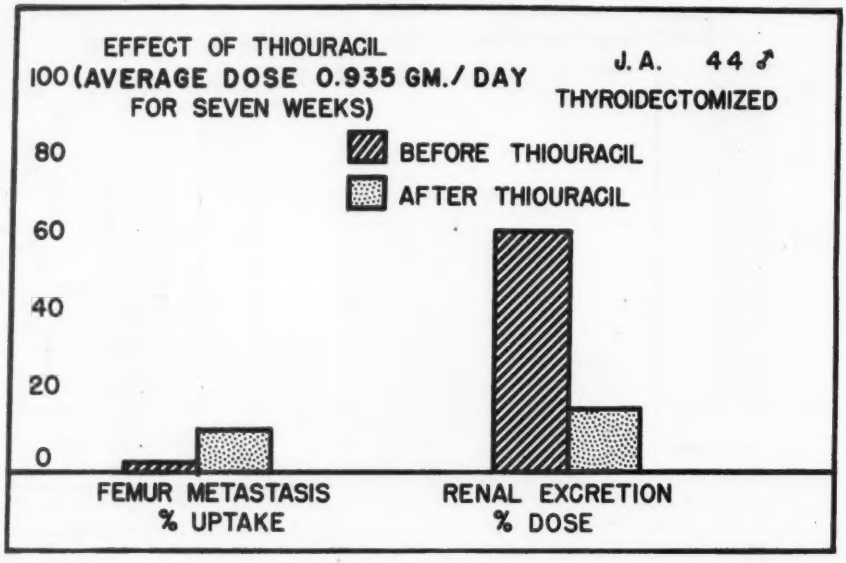

FIG. 3. *The effect of prolonged treatment with thiouracil on the iodine-collecting capacity of metastatic cancer of the thyroid. Mr. J. A., M. H. #77311.*

The cross-hatched columns represent observations on the "pickup of radioiodine" by 1 metastasis and the urinary excretion of radioiodine before beginning treatment with thiouracil. The stippled columns represent the "pickup of I^{131}" by the same metastasis and the urinary excretion of I^{131} after prolonged treatment with thiouracil.

1946 an incomplete pathologic fracture of the right intertrochanteric femur was sustained. This healed with immobilization.

Upon admission to the hospital, he was found to be in a euthyroid state with an asymmetric goiter, over which there was considerable tanning from previous X-ray treatments. He was found to have demonstrable metastases in the intertrochanteric femur on the right, in the twelfth thoracic vertebral body, and in the soft tissues overlying the right wing of the sacrum. Tracer studies with I^{131} revealed a significant, but not adequate, capacity to concentrate iodine in all three metastases.

At this time it was decided to determine the effect of thyrotropic hormone on the collection of iodine by the thyroid and by the three metastatic lesions. Thyrotropic hormone was administered in daily doses of 30 mg. for eight days. Two days following the last injection, another tracer dose of I^{131} was administered. By in vivo measurements

made forty-eight hours after administering the I^{131} the thyroid was found to have practically no increased avidity for iodine. All of the metastases, however, were found to have a two- to fourfold increase in capacity for collecting iodine (Fig. 2).

A third test dose of radioactive iodine administered eighteen days after stopping treatment with thyroid-stimulating hormone revealed a complete failure to concentrate iodine by the thyroid and all metastases. Recovery from this refractory state required several weeks.

Total thyroidectomy was finally done on this patient on November 17, 1948. Seventy days following total removal of the normal thyroid and the primary tumor, the patient was still in a euthyroid state. A test dose of I^{131} administered at that time was handled as follows: 63.4 per cent of the dose was excreted in the urine during a ninety-six hour period; in vivo measurements over the neck revealed no functioning thyroid tissue; and over the femoral metastasis it was shown that 3.6 per cent of the dose had been picked up by that lesion.

On February 19, 1949, the patient started taking thiouracil, 1.5 Gm. daily. This dosage had to be reduced to 0.9 Gm. daily because of vertigo. During an eighty-one-day period ending May 14, he received a total of 75.7 Gm. During twenty days of this period he received no thiouracil. The average daily intake was, however, 0.935 Gm. per day.

On May 16 (forty-eight hours after thiouracil was stopped), the patient was given a test dose of I^{131} and was found to excrete only 17.2 per cent in the urine, while collecting 11 per cent in the femoral lesion (Fig. 3). On the basis of these alterations in the iodine-collecting capacity of his tumors, therapy was instituted with a dose of 125 mc. on May 20.

The methods of increasing the iodine-collecting capacities of metastatic cancer of the thyroid described above have been of practical importance because they have enabled us to localize therapeutic amounts of radioactive iodine in tumors which otherwise would not have been treatable with this agent. They have been equally important in the formulation of newer concepts of the physiology of such cancers. From these studies we have learned that certain cancers of the thyroid are capable of responding to the organism's need for thyroid hormone by assuming the function of the parent organ. We have also learned that such cancers are capable of responding to the thyrotropic hormone. These facts would indicate that these cancers, at least, are not wholly autonomous, but can be influenced by certain normal physiologic mechanisms.

Treatment of metastatic cancer of the thyroid with I^{131}

Nine patients having functional metastatic cancer of the thyroid have been subjected to intensive treatment with radioactive iodine. The total cumulative doses administered to these patients have varied between 144 mc. and 906 mc. The largest single quantity given to one patient has been 315 mc. At the present time, all of these patients have viable cancer. However, in 4 of our treated patients definite improvement, as evidenced by a decrease in tumor mass or by distinct histologic signs of tumor de-

struction, has been observed. This improvement is illustrated by the following case history:

D. S. (*M. H. #84479*), a 42-year-old Greek housewife, was admitted to Memorial Hospital on January 30, 1947. She had first noticed blurring of vision in the left eye late in 1944. This progressed to loss of vision in the lower field by the spring of 1945. At that time she began complaining of pain, first in the right knee, then in the back, shoulder and left jaw. She was admitted to another hospital in April 1946, where X-ray examination revealed osteolytic lesions in the lower femur and upper tibia. A biopsy of the

FIG. 4. *Skull roentgenogram on admission to hospital. Mrs. D. S., M. H. #84479.* Attention is called to the osteolytic lesions in the occiput, right temporal bone, and right frontal bone.

femoral lesion was performed and revealed a follicular adenocarcinoma of thyroid origin. During this admission she underwent a right hemithyroidectomy for removal of the primary tumor.

On admission to Memorial Hospital eight months later, the patient was found to be completely blind in the left eye and nearly so in the right. She was persistently nauseated, vomited frequently, was dizzy, and preferred to stay in bed. She complained of widespread skeletal pain. X-ray examinations revealed metastatic lesions in the occiput, right temporal bone, sphenoid, right frontal bone (Fig. 4), right mandibular ramus, right

humerus, the first and second thoracic vertebral bodies, right distal femur, and proximal tibia. Several nodular densities were also seen in the lungs. The lesions in the flat bones of the skull and in the mandible were approximately the size of lemons. There was marked left exophthalmos. The disc of that eye was somewhat atrophic. The right optic disc exhibited a papilledema of three or four diopters. The spinal fluid pressure was 550 mm. of water.

A preliminary tracer dose of I^{131} was given on February 11, 1947, and disclosed small concentrations of radioactivity in all except one of the metastases measured. The occipital lesion collected 1.5 per cent of the administered dose. A 63 mc. dose was administered thereafter in the hope that the remaining normal thyroid tissue might be destroyed. One month later, on March 19, 1947, a test dose indicated an eightfold increase in iodine collection by the occipital metastasis. The fraction of the dose collected by the thyroid itself, however, was very small. (This applied as well to all subsequent doses.)

Seven therapeutic doses ranging in size from 60 to 315 mc. (she has received a total of 906 mc.) were administered during the next fifteen months. Nearly fatal increases in cerebrospinal fluid pressure occurred after the first two such doses. However, radiation thereby delivered, caused sufficient regression in the skull lesions to permit the remaining quantities of I^{131} to be taken without the same difficulty. After a period of menorrhagia and metrorrhagia, menstruation ceased, the last period beginning on December 10, 1947.

In general, only one metastasis at a time collected damaging amounts of radioactive iodine. After its regression, another would assume the role of "major collector." There was observed, therefore, a "procession" of singly avid metastases, each of which received a relatively large amount of radiation from one or more of the therapeutic doses of I^{131}. Regression of tumors in the skull proceeded to a point of impalpability. This process was accompanied by disappearance of signs and symptoms of increased intracranial pressure and by return of vision in the right eye. There was also restoration of a small amount of vision in the left (previously exophthalmic) eye. A biopsy of the mandibular lesion was performed on June 2, 1947, and showed changes described by Dr. Frank Foote as indicative of early radiation damage (Fig. 6. Compare with Fig. 5, a section from the pre-treatment biopsy of the distal femur). Subsequent biopsies were done at the sites of the frontal and occipital lesions in March, 1949. These showed striking and incontrovertible evidence of tumorcidal effect (Fig. 7). Old follicular outlines were seen represented by fibrous remnants surrounded by a homogeneous matrix which resembled granular cartilage. An occasional swelled cell or group of cells were seen with no visible means of vascular support. The metastases from which these sections were taken are probably incapable of further aggressive action.

Because of the large number of metastases in this patient, and because not all were functional simultaneously, not all have been similarly destroyed. Indeed, this patient still has considerable active disease which is still under treatment. However, there has been undeniable improvement as a result of this form of treatment.

Toxic effects: In treating these patients with radioactive iodine in the doses that we have administered, certain toxic effects of a serious nature have been observed. These we label debits.

In 3 patients who had received large doses of I^{131} given for the purpose of destroying the normal thyroid or functioning metastases, clinical symptoms and signs of hyperthyroidism appeared within two weeks after the radioactive iodine was administered. In all of these individuals the diag-

nosis of hyperthyroidism was verified by elevated serum precipitable iodines (Fig. 8). In 1 of these patients, the first in whom the phenomenon

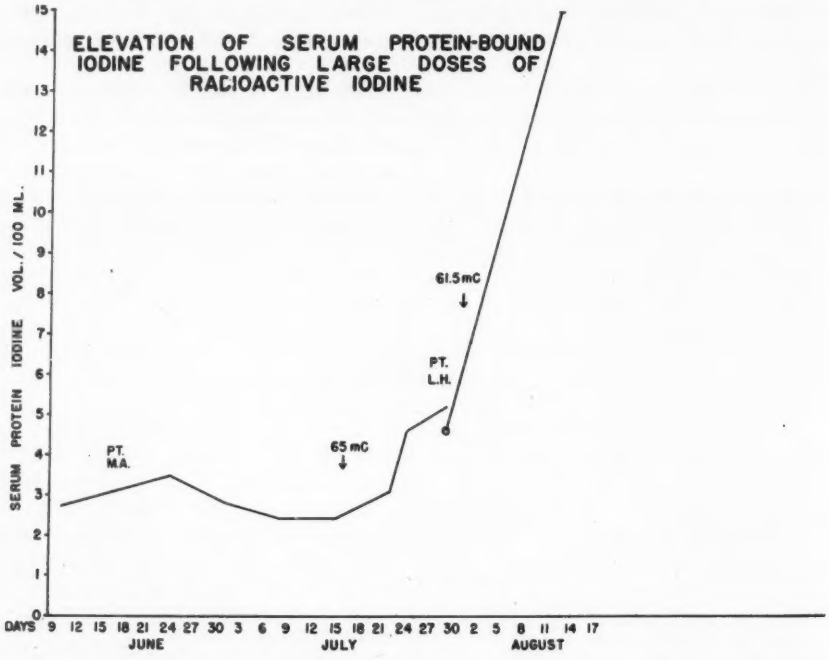
ELEVATION OF SERUM PROTEIN-BOUND IODINE FOLLOWING LARGE DOSES OF RADIOACTIVE IODINE
SERUM PROTEIN IODINE VOL. / 100 ML.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PT. M.A.
65 mC
61.5 mC
PT. L.H.
DAYS 9 12 15 18 21 24 27 30 3 6 9 12 15 18 21 24 27 30 2 5 8 11 14 17
JUNE
JULY
AUGUST

FIG. 8. *Elevation in serum precipitable iodine following large doses of I^{131} administered to destroy normal thyroid tissue.*

was observed, no pretreatment serum precipitable iodine level was obtained. We believe that this change resulted from breakdown of thyroid tissue and subsequent release of thyroglobulin.

Three female patients under the age of 41, who prior to receiving

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FIG. 5. *Histologic section of biopsy specimen taken from femoral lesion before any treatment. Mrs. D. S., M. H. #84479.*

Structure is that of follicular adenocarcinoma.

FIG. 6. *Histologic section of biopsy specimen from mandibular lesion three and one-half months after administering the first dose of I^{131}. Mrs. D. S., M. H. #84479.*

Attention is called to the pale edematous cells.

FIG. 7. *Histologic section taken from occipital lesion two years after first treatment with radioactive iodine. Mrs. D. S., M. H. #84479.*

Attention is called to the homogeneous matrix spotted by only occasional remnants of thyroid follicles.

FIG. 5

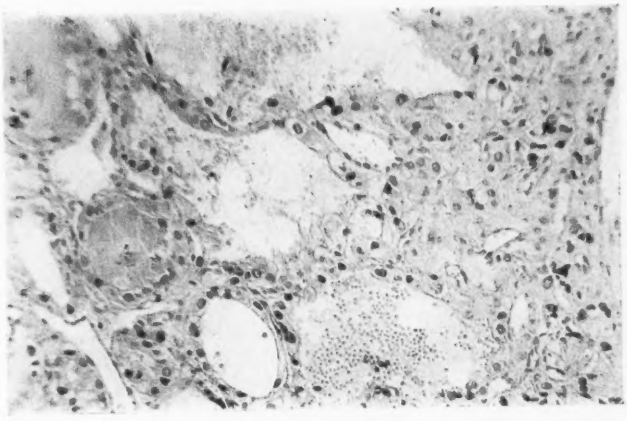

FIG. 6

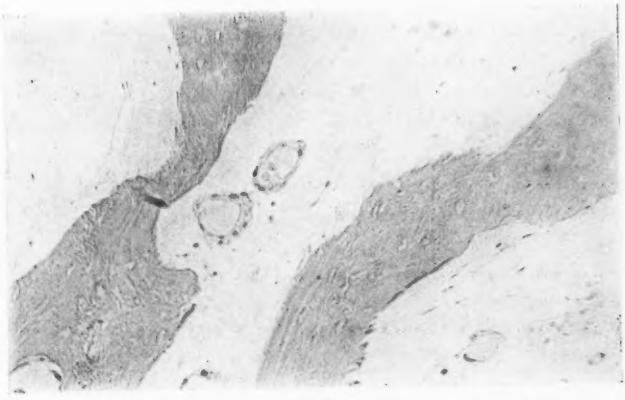

FIG. 7

radioactive iodine had normal menstrual cycles, developed amenorrhea after receiving cumulative doses of 90 to 458 mc. The estimated total blood radiation in these patients before the development of amenorrhea was equivalent to 200, 400, and 450 roentgens. Following the development of amenorrhea all of the patients complained of moderate to severe hot flashes. In 2 of these patients, the follicle-stimulating hormone titer in the urine was determined quantitatively several times. All of the results were within the normal range. Since the urinary follicle-stimulating hormone was not decreased in the 2 patients that we have studied, we do not feel that this change in menses can be attributed to destruction of the pituitary by irradiation from I^{131}.

Although it has been reported that the ovary contains iodine (6), we have been unable to demonstrate that the ovary of the rat collects radioactive iodine. Furthermore, we have been unable to demonstrate this isotope in the ovaries of adult women examined at varying intervals after administering radioactive iodine (7). Therefore, we have attributed these changes in the menstrual cycle to damage to ovarian tissue from circulating radioactive iodine.

The most serious side effect that we have observed in patients being treated for cancer with radioactive iodine has been depression of the hematopoietic tissues. Of the 9 patients treated with radioactive iodine in therapeutic doses for metastatic thyroid cancer, all were observed to have a depression of one or more peripheral blood elements.

The earliest change seen in most of those patients receiving the least amount of radiation to the blood has been an almost immediate fall in the absolute number of circulating lymphocytes to a level of one-third to one-fourth the normal count. Later, there has been a transient fall in blood platelets (Fig. 10). In one patient, this fall in blood platelets was associated with the appearance of petechiae. About two months after administering radioactive iodine in therapeutic doses, a small but significant decrease in the hemoglobin level was observed. In line with these observations, Koch, Talbot, and Bernstein (8), of our clinic, have observed that patients who have received doses as small as 10 mc. of I^{131} have an increased urinary and fecal excretion of coproporphyrin (I and III) which occurs between ten and fourteen days after administering the I^{131}.

In 1 patient whose cumulative dose of administered I^{131} was 638 mc., a fatal pancytopenia developed. Her history follows:

P. G. (M. H. #84972), a 38-year-old married Jewish female musician, was admitted to Memorial Hospital on March 17, 1947. She had undergone a subtotal thyroidectomy in 1940 for what was said to have been an adenoma. She remained well until December 1946, when parasthesia, followed by pain, appeared in the right mandibular region. A mass appeared in the subparotid area and rapidly increased in size. A biopsy on March

21, 1947, was reported to show metastatic alveolar adenocarcinoma of the thyroid with a few follicular components. Slides were obtained from the thyroid adenoma originally removed and the primary cancer was identified therein.

Further X-ray examination revealed additional metastases in the lungs, right femur, lumbar spine, and sacrum.

There were several episodes of massive atelectasis related to obstruction of the left lower bronchus by a metastasis. Symptoms were relieved by X-ray therapy to the lung metastases. The mandibular and femoral metastases were similarly treated. Despite this therapy, however, these and the other bone lesions continued to grow rapidly. Preliminary tracer studies indicated measurable but completely inadequate collection

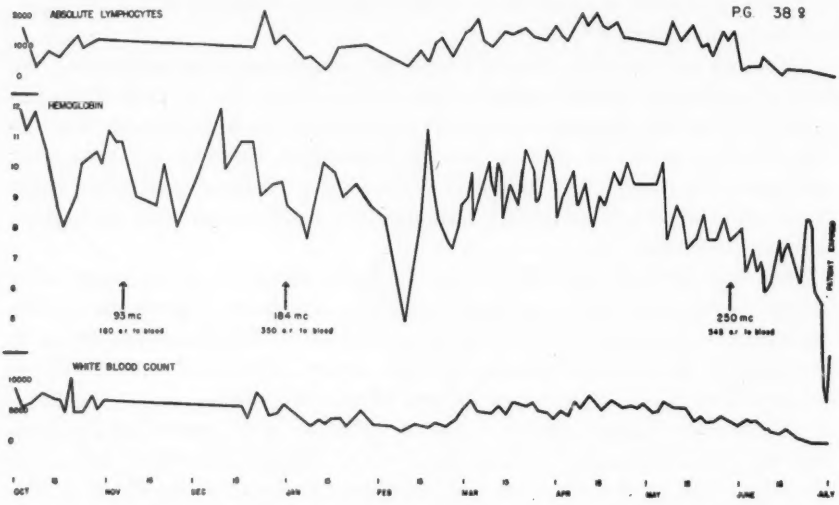

FIG. 9. *Changes in formed elements of peripheral blood in one patient who developed a fatal pancytopenia following treatment with a total of 588 mc. of* I^{131}. *Mrs. P. G., M. H. #84972.*

of radioactive iodine by the mandibular and femoral metastases. Accordingly, a dose of 61 mc. was administered on April 10, 1947, for the purpose of destroying remaining thyroid tissue.

Although there were increases in iodine-collecting capacity by factors of 200 (mandible) and 50 (femur), the urinary excretion remained consistently high. However, because of the rapidity with which metastases of this poorly differentiated tumor were growing, therapy with radioactive iodine was undertaken.

By September 1947, the patient was clinically myxedematous (serum cholesterol 275 mg. per cent, serum precipitable iodine 1.2 micrograms/100 ml., and BMR −32 per cent). Menstrual irregularity and profuse, prolonged flow began in August 1947. A histologic diagnosis of hyperplastic endometrium was made following endometrial biopsy. Menstruation ceased in mid-September and never recurred.

Therapeutic quantities of I^{131} were administered as follows: 93 mc. on November 6, 1947; 184 mc. on December 31, 1947; and 250 mc. on May 29, 1948. This constituted

heroic therapy because of relatively low iodine uptake by the tumors, but was felt to be justified after consideration of rapid tumor growth and of the unavailability of other therapeutic techniques. Figure 9 illustrates changes in the peripheral blood elements following each therapeutic dose. The fall in hemoglobin after the second (184 mc.) dose was correlated with a massive hematuria which ceased spontaneously. Subsequent to the third (250 mc.) dose, however, there was rapid depletion of all formed elements in the blood, and the patient died thirty-five days later.

Postmortem studies indicated that no detectable radiation changes had been induced in the metastases, which involved bones, lungs, kidneys, adrenals, liver, and heart. It has been estimated that the blood radiation from the total of 638 mc. of I^{131} administered to this patient was 1,300 equivalent roentgens.

Bone marrow aspiration biopsy specimens were studied by Dr. J. B. Thiersch before and at various intervals after administering radioactive iodine in therapeutic doses in 5 patients. In 2 more patients, whose bone marrows were not examined before treatment, two or more bone marrow biopsy specimens were studied after treatment with therapeutic amounts of radioactive iodine. In all of these post-treatment marrow examinations there has been observed a uniform decrease in the total cell count. This has been accompanied by a reversal of the erythroid myeloid ratio. That is, there was observed a relative increase in the erythroid activity and a decrease in myeloid elements. In patients whose blood was exposed to lower levels of irradiation, as determined by the level of radioactivity in the blood at varying intervals after administering the I^{131}, there has been observed a tendency for the marrow to recover within a period of four to six months. These changes are illustrated in the following case history:

W. M. (*M. H. #87060*), a 15-year-old white male, was admitted to Memorial Hospital in May 1947. He had been under constant close supervision in another institution because of severe rheumatic heart disease. In the course of investigation of repeated pulmonary hemorrhages, X-ray examination revealed miliary nodular densities throughout both lung fields. These were at first thought to represent either tuberculosis or some other infection. However, enlarged cervical nodes were biopsied and found to contain a low-grade follicular adenocarcinoma of thyroid origin. A preliminary tracer study indicated no measurable uptake in the lung metastases. Accordingly, 25 mc. of I^{131} was given on September 19, 1947, to destroy the function of the normal thyroid gland. Following a test dose on April 7, 1948, it was evident that significant quantities of iodine were being incorporated by the pulmonary lesions.

Under ordinary circumstances the patient might then have been treated with a therapeutic dose of radioactive iodine. However, it was feared that breakdown of tumor might produce transient thyrotoxicosis which would impose too great a burden on his badly damaged heart. Prolonged treatment with thiouracil was undertaken in an effort to deplete the metastases of stored "hormone" prior to the administration of I^{131}.

Although the patient weighed only 98 pounds and received doses of thiouracil as large as 1.5 Gm. daily for ten months, there was never any evidence of hypothyroidism. A test dose of I^{131} was given December 13, 1948, forty-eight hours after stopping the

administration of thiouracil. Only 4.3 per cent of the dose was excreted in the urine in ninety-six hours. This unusually low excretion was verified by another tracer dose. On December 29, 1948, a therapeutic dose of 112 mc. was given. Figure 10 shows the changes which subsequently took place in the blood. Figure 11 illustrates results ob-

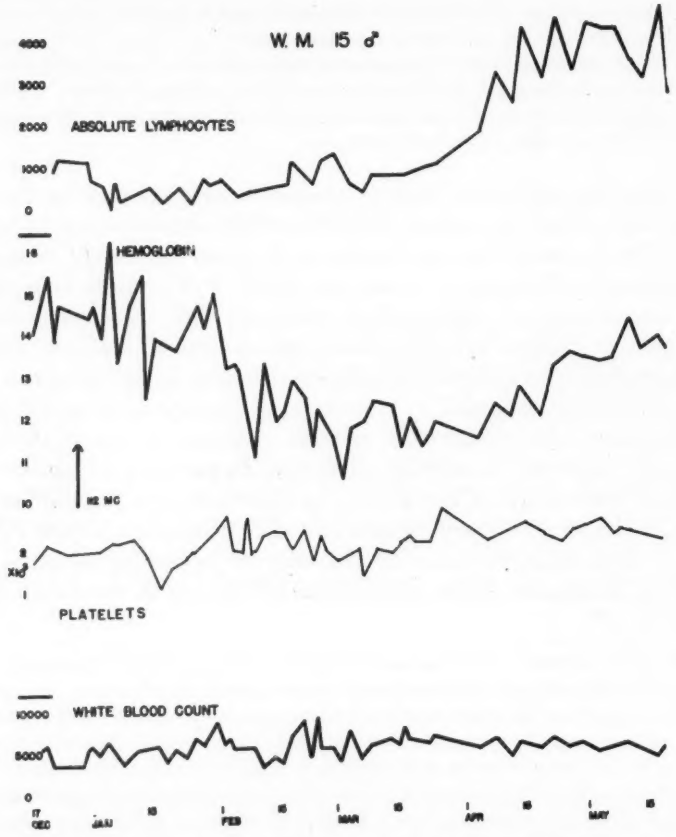

FIG. 10. *Changes in formed elements of peripheral blood in one patient following administration of 112 mc. of* I^{131}. *Mr. W. M., M. H. #87060.*

Attention is called to immediate drop in lymphocytes with subsequent recovery, and delayed drop in platelets and hemoglobin with subsequent recovery. Only six per cent of the I^{131} was excreted in the urine during 96-hour period.

tained from studying bone marrow aspirations before and during a period of three and one-half months after treatment. Tendency toward recovery is seen in both the peripheral blood and in the marrow. This case probably represents the ideal setting for delivery of large amounts of radiation to thyroid cancer metastases while exposing the hematopoietic system to an amount from which it can recover satisfactorily.

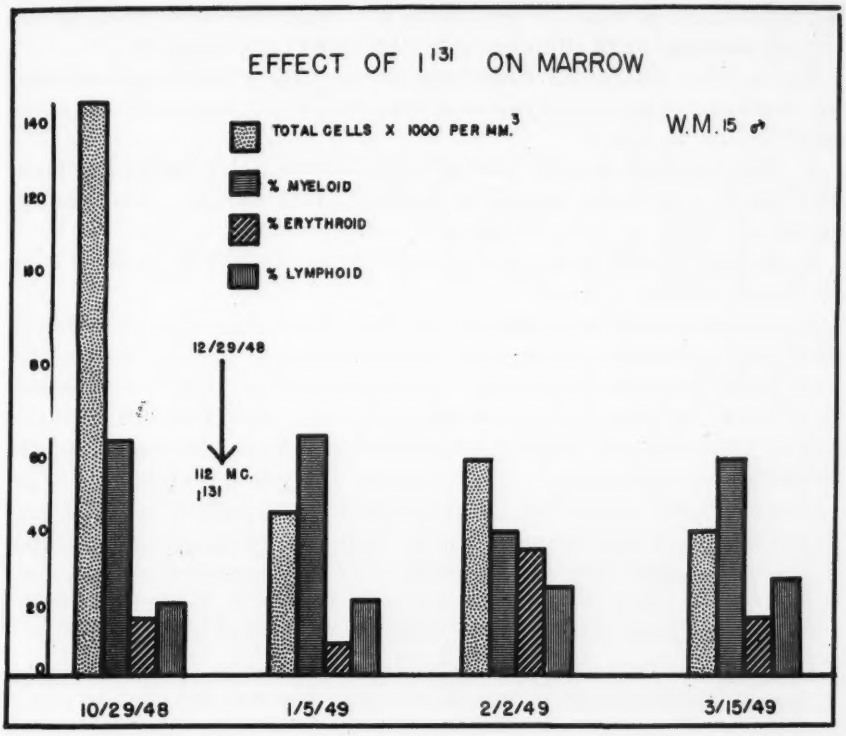

FIG. 11. *Changes in total cells, myeloid, erythroid, and lymphoid cells of marrow following administration of 112 mc. of I^{131} to patient who excreted only 6 per cent of administered radioiodine in 96-hour period. Mr. W. M., M. H. #87060.*

SUMMARY

We have summarized our experiences in treating metastatic cancer of the thyroid with radioactive iodine and have discussed them as credits and debits.

On the credit side are the following:

1. Definite clinical improvement has been observed in 4 out of 9 cases subjected to intensive treatment with the eight-day half-life radioactive isotope of iodine.

2. In these patients a tumorcidal effect as evidenced by shrinkage of tumor mass and by histologic evidence of cell destruction, has been observed.

3. We have learned from these studies certain aspects, hitherto unknown, of the physiology of thyroid cancer—that metastatic cancer of

the thyroid can be made to respond to physiologic needs of the body for thyroid hormone or to stimulation by the thyrotropic hormone.

On the debit side we have observed certain toxic effects resulting from the treatment of metastatic cancer of the thyroid with large doses of radioactive iodine, as follows:

1. Hyperthyroidism with elevated serum precipitable iodine has been observed in 3 euthyroid individuals having normal thyroids or functioning metastases, following the administration of large doses of I^{131}.

2. Amenorrhea has been observed in 3 women under the age of 41 who had received large doses of I^{131}.

3. A depression of hematopoiesis has been observed in all of the patients that we have subjected to intensive treatment with radioactive iodine. The toxic effects on hematopoiesis have been evidenced not only by decrease in one or more peripheral blood elements, but also by decreased cell counts and reversal of the erythroid myeloid ratios in bone marrow aspiration biopsies.

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THE FUNCTION OF VARIOUS TYPES OF THYROID CARCINOMA AS REVEALED BY THE RADIOAUTOGRAPHIC DEMONSTRATION OF RADIOACTIVE IODINE (I^{131})*†

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IN the six-year period from 1943 to 1949, there have been studied by the radioautographic technique at the Department of Pathology, Memorial Hospital, and the Department of Physics, Sloan-Kettering Institute, New York City, 100 specimens of carcinoma of the thyroid from patients who had been previously given radioactive iodine (I^{131}). In all cases the diagnosis of carcinoma of the thyroid was made from histologic sections by the Department of Pathology, Memorial Hospital, either from hospital or submitted material. The group comprises 94 operative specimens and one aspiration biopsy specimen from 81 patients, and tissue from the necropsies of 5 patients.

In 89 cases, "tracer" doses of iodine—varying from 0.3 to 5.0 millicuries of carrier-free I^{131}—were given to the patient, usually twenty-four to forty-eight hours before surgical biopsy of the lesion was performed. Nine patients received "therapeutic" doses of from 27 to 250 millicuries of the isotope, usually from two to ten days before operation. Two patients received 10 millicuries for investigative purposes. Histologic processing of the tissue generally required forty-eight hours from time of operation to exposure on the photographic emulsion. Patients given the smallest amounts—0.3 and 0.4 millicuries—have shown positive radioautographs, whereas patients given about 20 and 30 millicuries (allowing for decay) gave negative radioautographs. In the latter, the carcinomas were of a type that does not concentrate the isotope. For this reason it is believed

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that our "tracer" doses are adequate to demonstrate significant concentration of the isotope if the carcinoma is of the receptive type.

The first 50 cases were studied by the technique of Hamilton *et al.* (1) of placing a glass slide containing the histologic section of radioactive thyroid carcinoma against the emulsion surface of a photographic plate or roentgen film. The second 50 cases were studied by floating paraffin sec-

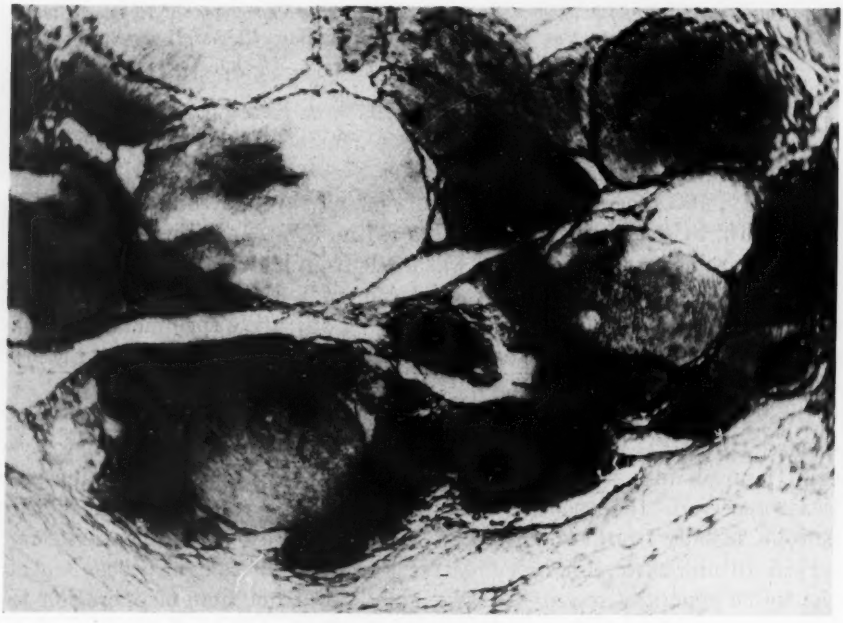

FIG. 1. Normal thyroid. Marked variation in the amount of radioactivity (dark areas) from follicle to follicle. Artefacts within follicle caused by loss, or folding, of colloid. ×200. NTB emulsion.

tions of tissue on to the emulsion surface of a photographic plate or roentgen film. This method is that of Evans (2) and Endicott and Yagoda (3). In this technique, tissue sections remain fixed to the photographic emulsion during the process of development and fixation of the film and during the staining of the tissue. Roentgen film and lantern slide plates were used in all cases and, recently, in 20 cases, Kodak nuclear track plates (NTB) as described by Boyd (4) have also been used. Exposure time has been estimated by measuring directly the tissue activity of the histologic section, or one cut a few microns away from the one used for exposure (the method of Marinelli and Hill (5)). Exposure was carried out in light-tight black

plastic slide boxes sealed with black photographer's tape and kept at ice box temperature (5–7°C.) throughout. Development and fixation of the film or plate were carried out by standard procedures. All tissues of the latter 50 cases were stained by a combination of metanil yellow and iron hematoxylin stains which are not as readily absorbed by the photographic

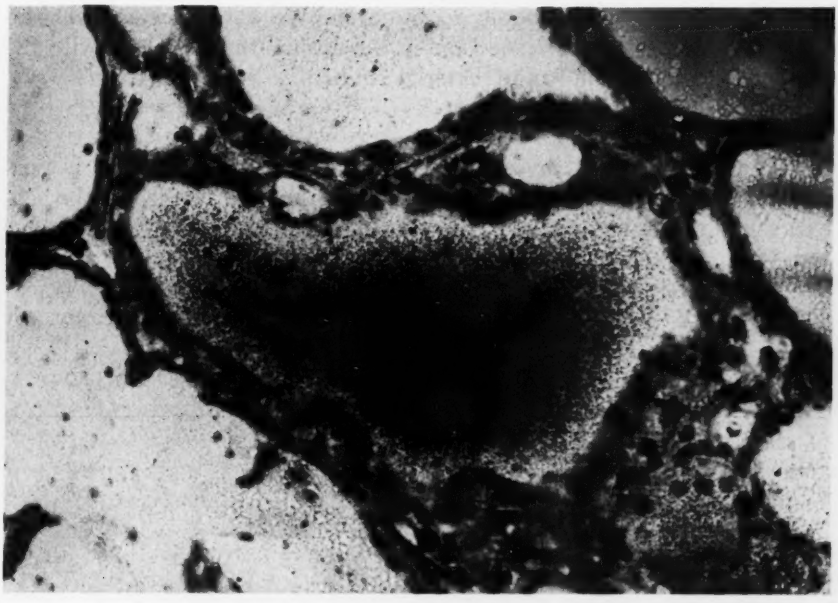

FIG. 2. High power view of a follicle with much radioactivity (dark granules) showing concentration of the isotope in the colloid of the follicles. Adjacent follicles show little or no radioactive substance. Lantern slide emulsion. ×600.

emulsion as are hematoxylin and eosin. There is also less tendency for the metanil yellow and hematoxylin to mask minimal amounts of reduced emulsion granules (6) (7). Marinelli, Foote, Hocker and Hill (8) reported on the first 19 cases in this group and, later, one of us analyzed 47 of these cases (6).

Normal thyroid gland

The normal thyroid gland shows by radioautography many interesting features (Figs. 1 and 2). The most conspicuous is the marked variability in the amount of radioactive iodine that is concentrated in the follicles. Many follicles appear to contain little or no radioiodine; others show a heavy concentration. Adjacent follicles may vary from one extreme of no evi-

dence of radioactivity to the other of a very heavy uptake of the isotope. Frequently the smaller follicles contain the highest concentration of isotope and may sharply contrast with an adjacent large follicle which has concentrated little or none. In all our human surgical and autopsy material there has been a gap of at least twelve to twenty-four hours from the time of administration of the isotope to the patient until the thyroid tissue was exposed on the photographic emulsion. We have seen evidence of radioactivity only within the colloid in the normal thyroid gland with the present techniques and in the material studied.

Thyroid carcinoma

The classification of thyroid carcinoma at Memorial Hospital is relatively simple. The groupings show some overlapping of types but the general classification is a workable one (Tables 1 and 2).

TABLE 1. RADIOAUTOGRAPHIC CONCENTRATION OF I^{131} IN THYROID CARCINOMA—100 CASES

Type	Number of cases	+	−	%+
Papillary	29	8	21	28
Alveolar and follicular	39	29	10	74
Solid	12	5	7	42
Hürthle cell	9	3	6	34
Giant and spindle cell	6	0	6	—
Anaplastic	3	0	3	—
Unclassified	2	1	1	—
Total	100	46	54	46

TABLE 2. SEPARATE LESIONS STUDIED

Type	Primary			Metastatic			Total		
	No.	+	%+	No.	+	%+	No.	+	%+
Papillary	35	4	11	42	16	38	77	20	26
Alveolar and follicular	46	31	67	65	54	82	111	85	77
Solid	12	6	50	16	5	31	28	11	39
Hürthle	13	2	15	6	1	13	19	3	16
Giant and spindle cell	11	0		3	0		14	0	
Anaplastic	1	0		5	0		6	0	
Unclassified	1	0		2	2		3	2	
Total	119	43	36	139	78	56	258	121	47

Papillary carcinoma is the most common type, the one showing the best prognosis, and the type often occurring in young patients. About 50 per cent of our thyroid carcinomas fall into this group (9). Small buds or papillae of vascularized connective tissue are outlined by a layer or two of cuboidal or columnar epithelial cells at the periphery of the stalks of connective tissue. For the purpose of radioautographic discussion we have subdivided this type into two groups. True gland formation and colloid make up less than 15 per cent of the tumor in about one-third of our cases and this type we have called, *predominantly papillary*. Even in this group other types are usually present, although in minimal amounts. Those papillary tumors containing chiefly a papillary component but also 15 to 40 per cent of other features, we have called, *mixed type*.

There were 29 cases of papillary carcinoma. Eleven of these were nearly uniform throughout, and 18 showed admixtures of various types, usually alveolar formation and/or follicle formation with colloid. Of the 11 *predominantly papillary* forms none showed any uptake of radioactive iodine in primary or metastatic lesions even though, in all cases at least 0.5 millicuries, and in one case 59.9 millicuries, of I^{131} were given. In 7 cases adjacent, normal thyroid tissue showed retention of the isotope. A total of 29 lesions (15 primary and 14 metastatic) were studied in this group (Table 2).

Of the 18 cases in which there were areas showing colloid or alveolar formation, 10 did not show the presence of radioactive iodine either in primary or metastatic lesions. From 0.4 to 3.1 millicuries of I^{131} were given in this group, and 6 cases with normal adjacent thyroid tissue showed concentration in the normal thyroid. Twenty-eight separate lesions (16 primary and 12 metastatic) gave negative results.

In 8 cases concentration of radioactive iodine was evident either in the primary or metastatic lesions. Six of these 8 cases showed positive radioautographs in the metastatic areas, 4 gave evidence of activity in the primary lesion, and 2 cases gave positive results in both the radioautographs of the primary and metastatic lesions. The lowest dose (0.3 millicuries) given in the papillary groups showed concentration in the carcinoma.

Thus, in 29 cases (77 lesions) of papillary carcinoma 21 cases (57 lesions) showed *no* evidence of concentration of radioactive iodine. The 8 cases (28 per cent) (20 lesions) that did give evidence of such, contained histologic variation from a *predominantly papillary* form, *i.e.*, they demonstrated either follicle or alveolar formation, or both (Fig. 3).

Follicular and alveolar carcinoma comprises about 10 per cent of the carcinomas of the thyroid occurring at Memorial Hospital (9). It is distinctly more malignant clinically than the previous group. Histologically these tumors are characterized by follicle formation—reproduction by

tumor tissue of some replica, however distorted, of the thyroid follicle—with an admixture of glandular structures composed of epithelial tumor tissue having some resemblance to thyroid epithelium. The alveolar component possesses a lumen empty of colloid as far as can be determined by routine histologic methods. We include so-called metastasizing struma in

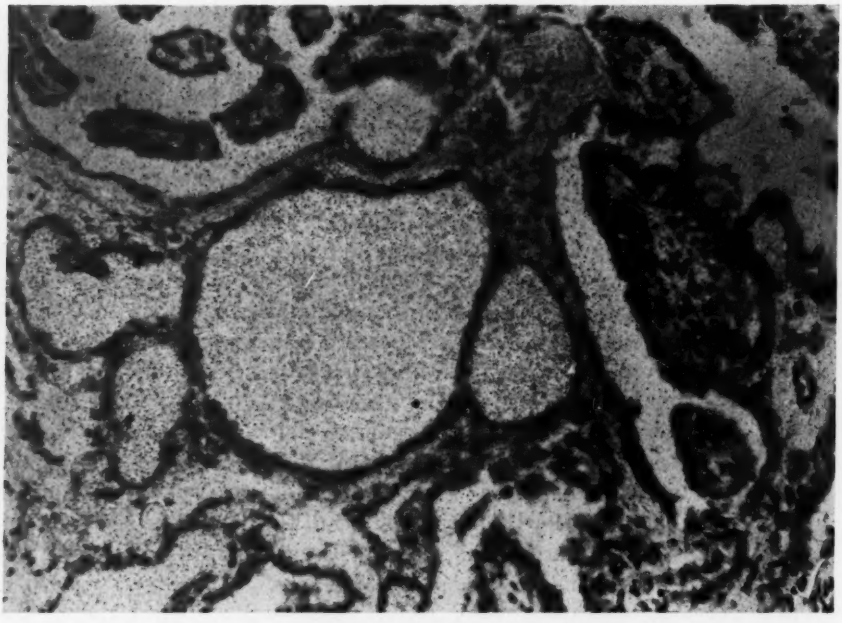

FIG. 3. Papillary adenocarcinoma of the thyroid with a few areas containing colloid which show minimal amounts of radioactivity (as shown by increase of reduced emulsion granules over background). Only these areas pick up the isotope; the papillary portions do not. Lantern slide emulsion. ×120.

this group inasmuch as careful search of many lesions usually reveals alveoli without colloid, or areas of solid carcinoma.

Thirty-nine cases of alveolar and follicular carcinoma of the thyroid have been studied (Table 1). Twenty-nine (74 per cent) showed evidence of concentration of the isotope by radioautography and 10 gave negative results. Of 21 cases (46 lesions) with primary lesions, 15 cases (31 lesions) gave positive findings (Table 2).

Alveolar areas, as a rule, did not tend to pick up the iodine (Fig. 4). Usually, the concentration of radioactive iodine varied directly as the ratio of colloid to the total amount of tumor tissue present. However, there

was a marked variation in the concentration of the isotope in the tumor, just as in normal thyroid (Figs. 5 and 6). This type, obviously, offers the best possibilities for therapeutic use of radioiodine and it may be stated as a general rule that the more differentiated the tumor—the more closely it resembles the normal thyroid follicular pattern—the greater the likelihood of its concentrating radioiodine.

One outstanding exception to the parallel presence of colloid and radio-

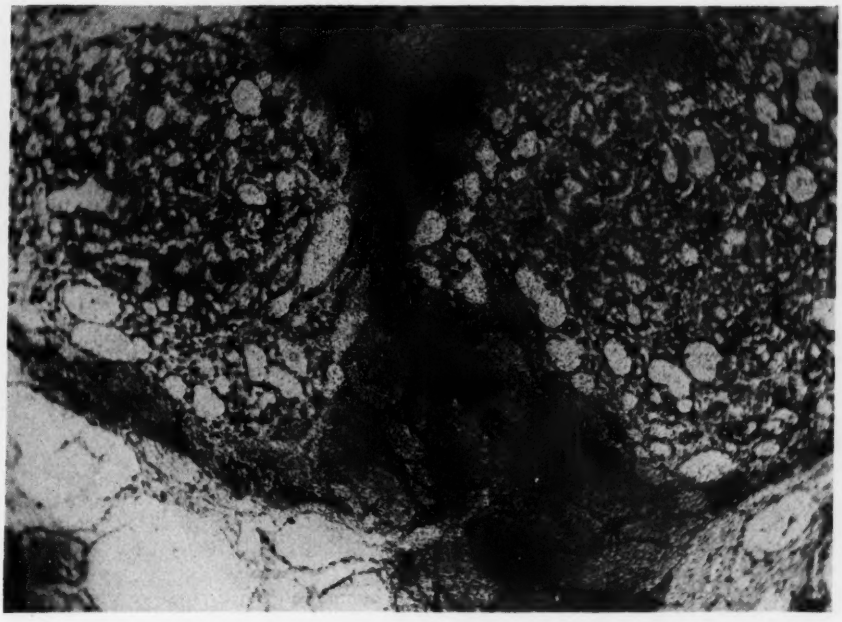

FIG. 4. Two foci of follicular and alveolar carcinoma in the thyroid gland. The carcinoma does not show radioactivity, but the adjacent intact normal thyroid follicles pick up the isotope in varying amounts. Lantern slide emulsion. ×120.

active iodine was in a patient whose metastatic lesion was composed of alveolar formation without any demonstrable evidence of colloid and yet retention of I^{131} occurred. In all, 111 distinct lesions were studied and 85 (77 per cent) were shown to have concentrated the isotope (Table 2).

Solid carcinoma is a type characterized by clumps of cells without the presence of a significant lumen. The cells grow in solid sheets and frequently show considerable nuclear variation. The tumor makes up about 20 per cent of the thyroid carcinomas in the cases at Memorial Hospital (9). Twelve cases (28 lesions) of this variety were studied (Tables 1 and 2).

For the purposes of radioautographic study it was decided to classify the cases histologically into two types, independently of any knowledge of the radioautographic results, to determine whether, as in the papillary group, any correlation between the histologic types and the concentration of radioiodine occurred. Any lesion showing 85 per cent or more of the solid

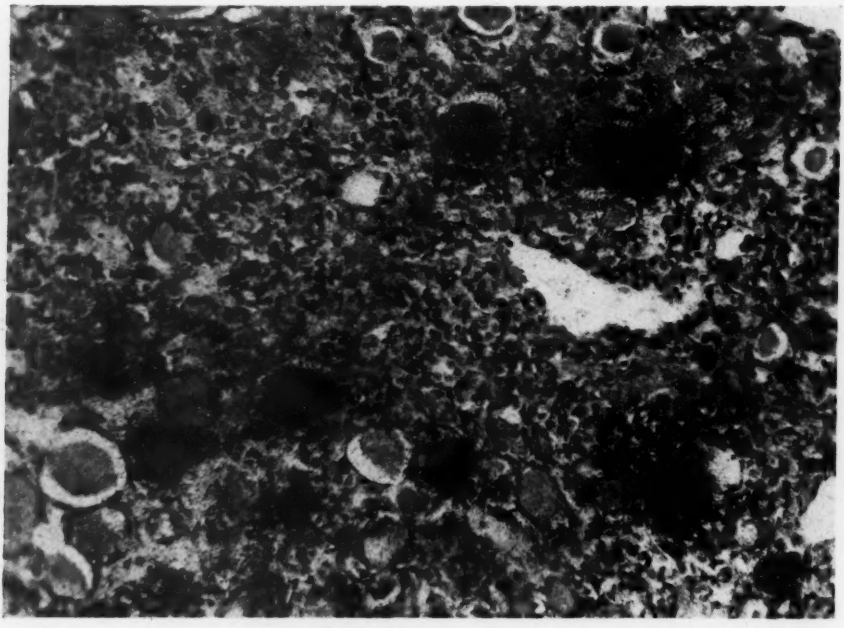

FIG. 5. Metastatic alveolar and follicular carcinoma of the thyroid showing radioactivity (dark areas) in some, but not all, of the follicles. Lantern slide emulsion. ×120.

type was called, *predominantly solid.* Those lesions chiefly solid but containing admixtures of alveolar, follicular, or papillary areas were called, *mixed.*

Eight cases (18 lesions) were of the *predominantly solid* type. One case concentrated the isotope in 2 metastatic lesions—one a cervical lymph node; the other in the lung. Seven cases (16 lesions) showed no localization of the isotope in the tumor, although in 3 of these cases normal adjacent thyroid showed positive findings. In the *mixed* variety, 4 cases (10 lesions) were examined. All 4 cases showed positive radioautographs; in some, one or more of the primary or metastatic lesions and 9 of the 10 lesions studied, gave positive results.

Thus of a total of 12 cases (28 lesions), 5 cases (11 lesions) had positive

radioautographs. In this type, also, there was great variability in concentration of the isotope and often in the same low power microscopic field one could see pronounced differences in radioactivity in adjacent areas (Fig. 7). Two patients received about 5 millicuries of I^{131} without any radioautographic evidence of concentration of the isotope. In the *mixed type* the isotope was usually present in the alveoli (Fig. 8).

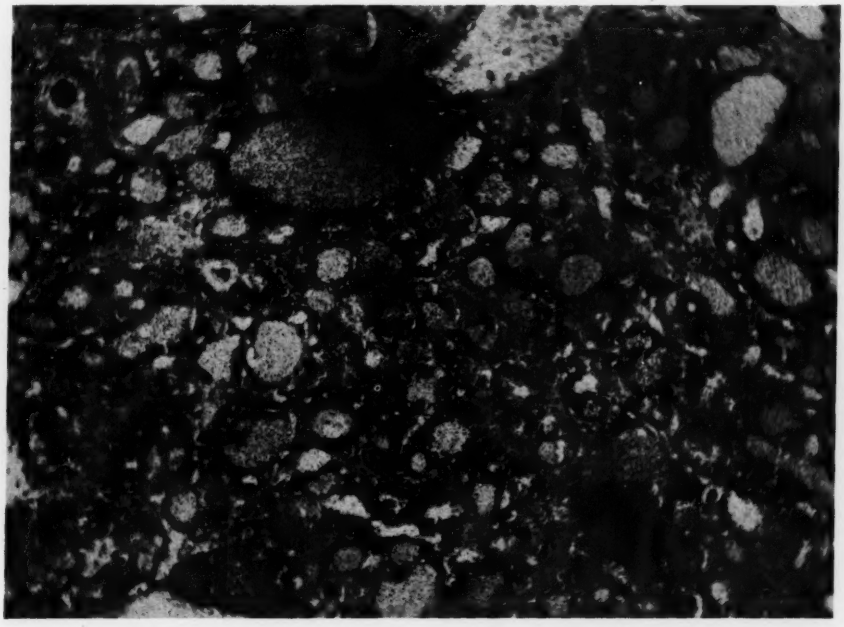

FIG. 6. Metastatic alveolar and follicular carcinoma of the thyroid. Focal concentration of radioactive I^{131} (dark areas). Lantern slide emulsion. ×120.

Hürthle cell carcinomas make up about 10 per cent of our thyroid carcinomas (9). They are characterized by bright-pink-staining, abundant cytoplasm in the usual hematoxylin and eosin stained slide. The cells frequently grow in strands or columns in which cell boundaries are indistinct and the nuclear arrangement suggests stratification. However, other types such as a glandular, and a diffusely infiltrating, polygonal cell type, still showing the characteristic tinctorial cytoplasmic eosinophilia, are well known. The clinical impression, at present, is that these often run a fairly long course with late metastasis, although exceptions to this impression are known.

Nine of these cases were examined with primary lesions available in 7 cases and metastatic in 3. Three cases showed a rare focal area of concen-

tration of iodine but only in 3 lesions—2 primary and 1 metastatic—out of 19 studied. Normal thyroid tissue adjacent to 4 primary negative lesions showed retention of radioactive iodine. One case had a metastatic lesion in which about 20 per cent of the tissue showed glandular formation by the Hürthle cells with the presence of a colloid-like substance, yet no radioactive pick-up was demonstrated in this case. The 3 positive lesions showed the isotope in alveolar areas and a poorly staining colloid-like substance

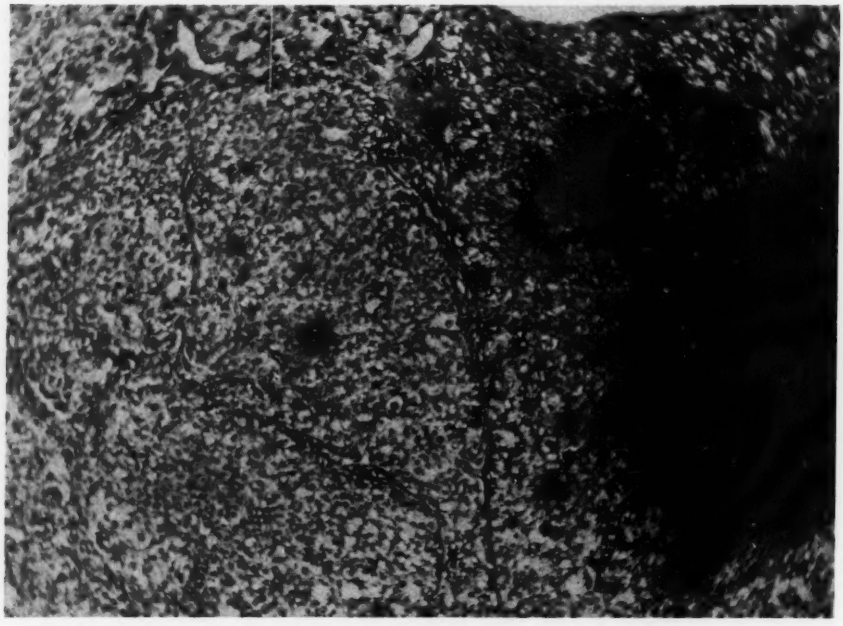

FIG. 7. Solid carcinoma of the thyroid—metastatic to cervical node. Variable concentration in the tumor. Lantern slide emulsion. ×120.

could be demonstrated in adjacent sections prepared by standard histologic techniques.

Dosage varied from 0.5 to 2.2 millicuries and the lowest dosage case was one of the ones concentrating the isotope.

Spindle and giant cell carcinoma has been the type proved to be the most malignant of our thyroid carcinomas. It makes up about 15 per cent of the total (9). Characteristically it shows anaplastic thyroid tissue growing in the form of spindle and giant cells resembling, at least superficially, sarcoma. Six cases (14 lesions) were studied. Primary lesions were available in 5 cases (11 lesions) and metastatic in 2 cases (3 lesions). All gave nega-

tive results. Occasionally the primary tumor, growing in the thyroid, circumscribed normal follicles that picked up the isotope and the false impression might have arisen that the tumor was concentrating the isotope (Fig. 9). Dosage varied from 0.9 to 2.0 millicuries. In 5 cases normal thyroid tissue was shown in the radioautograph as well as carcinoma. In all these cases the findings were positive in the thyroid tissue but negative in the tumor.

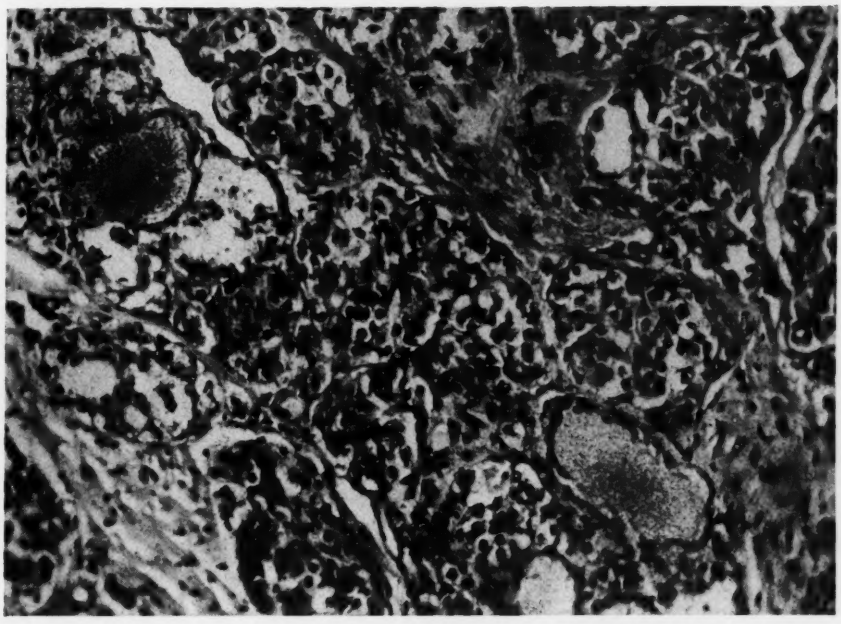

FIG. 8. Predominantly solid carcinoma of the thyroid—metastatic. Radioactivity demonstrated in a few large alveoli. Lantern slide emulsion. ×120.

Anaplastic carcinoma of the thyroid is a rare form making up about 1 per cent of the Memorial Hospital series. The cell type is small, the cellular infiltrate is patternless and often resembles tumors of the malignant lymphoma group. Three cases (6 lesions) were studied. The primary lesion of 1 case and 5 metastatic lesions of 2 cases were all negative. Dosages were 2.0, 3.2, and 62.2 millicuries. In the case with the lowest dose, normal thyroid tissue was present and showed concentration of the isotope.

Two cases were *unclassified.* One was an aspiration biopsy specimen from the thyroid gland of a patient who had received I^{131} forty-eight hours prior to the biopsy. The diagnosis of "carcinoma" was made because of the

presence of clumps of malignant cells but the radioautograph was negative. A subsequent biopsy and radioautograph showed alveolar and follicular carcinoma of the thyroid which did not concentrate the isotope.

One case of thyroid carcinoma with areas of solid, alveolar, and follicular elements in the primary and metastatic areas but, in addition, containing giant cells, bone formation, spindle-shaped cells, monocytic and lympho-

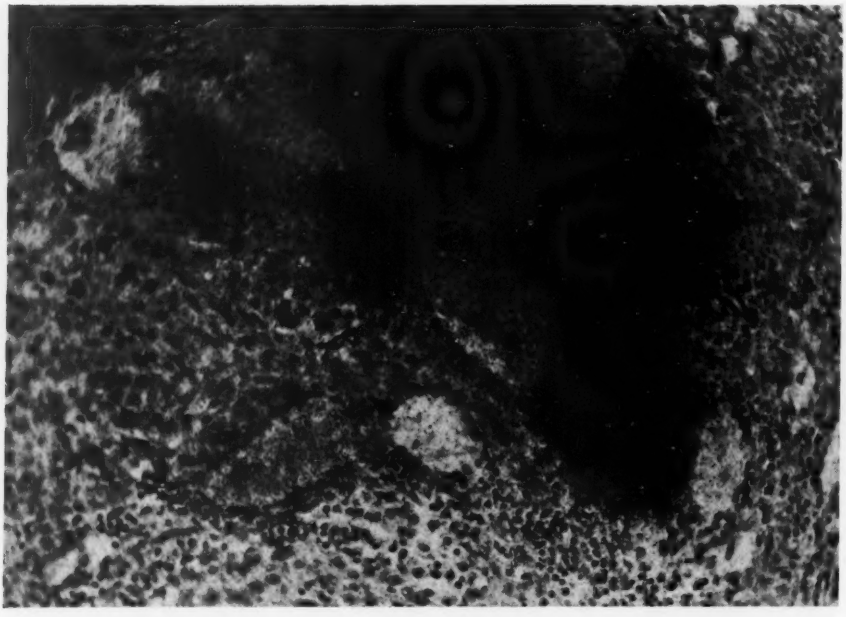

FIG. 9. Giant and spindle cell carcinoma of the thyroid. No radioactivity in the tumor but heavy concentration of the isotope in the remaining intact thyroid follicles. Lantern slide emulsion. ×120.

cytic infiltration was unclassified. There was concentration of I^{131} in the follicular areas of the 2 metastatic lesions studied. The dose was 3.0 millicuries.

DISCUSSION

It has been shown that 46 of 100 cases of thyroid carcinoma from selected patients who had previously been given I^{131} concentrated the isotope enough to give positive radioautographic evidence of localization in the carcinoma tissue (Table 1). This group represents 258 separate lesions from 82 patients. Of these, 121 (47 per cent) showed positive results. If our series and techniques are truly representative of the function of thyroid carcinomas, about one-quarter of the papillary group may be expected to

concentrate the isotope, at least enough to give radioautographic evidence of such. About three-fourths of the alveolar and follicular carcinomas of the thyroid in our series picked up the isotope. Solid carcinomas showed concentration of radioactive iodine in 5 of 12 cases, usually where follicular components were present but also where there were none. Although 3 out of a total of 9 Hürthle cell carcinomas showed a few foci of radioactivity, for all practical purposes, one would not consider our results encouraging in attempting to assess the avidity of these tumors for iodine. No evidence of activity was found in 10 cases of giant and spindle cell carcinoma, nor in 3 cases of anaplastic carcinoma of the thyroid.

Comparison of function of primary and metastatic lesions

Twenty five cases (113 lesions) were studied in which primary and metastatic lesions were excised simultaneously. In 22 (88 per cent) of these cases radioautographs of the primary and metastatic lesions were either both positive (11 cases) or both negative (11 cases). In one case the radioautograph of the primary lesion was positive and of the metastatic lesion, negative. Only one lesion of each was studied. In 2 cases the radioautograph of the primary lesion was negative and of the metastatic lesions, positive. It is believed that primary and metastatic lesions tend in general to concentrate radioactive iodine in similar fashion if the normal thyroid is present. Our figures show a much higher percentage of metastatic than of primary lesions concentrating the isotope (Table 2). However, these data might be misleading, as the factor of selection of material at the surgical desk, the influence of various types of treatment between removal of primary and metastatic lesions, and other factors confuse the issue.

Significance of colloid in carcinoma in relation to concentration of the isotope

It is obvious that concentration of the isotope is related primarily to the presence of colloid and that the relative ability of the various types of carcinomas to concentrate the isotope is paralleled by the amount of colloid present in the particular type. In so-called metastasizing struma, 17 different lymph nodes all concentrated the isotope. However, there must also be at work other very significant factors inasmuch as morphologically identical follicles, in normal as well as neoplastic thyroid tissue, vary tremendously in their concentrations of the isotope. The presence of colloid *per se* is no guarantee that radioactive iodine will be found in the tumor, as many follicles show no evidence of radioactive iodine whereas their neighbor follicles may contain considerable amounts. In 4 cases almost one-half of the tumor showed follicles and no concentration of the isotope was evident. We have seen the opposite—alveoli without colloid—concentrating the isotope in 3 cases.

Therapeutic considerations

From the point of view of the therapy of carcinoma of the thyroid with radioactive iodine, many questions immediately arise. Since there is no even, diffuse distribution of the isotope, could one ever hope to destroy or inactivate sufficient numbers of cells without having to give excessive amounts of radiation, if treatment were given in one or two large doses? One patient received a total of 570 millicuries of I^{131} in 5 separate courses in twenty-one months, yet at autopsy a few morphologically normal-appearing follicles were present in the thyroid area. Would repeated doses possibly reach more neoplastic cells because of some as-yet-unknown phasic variation of follicular function? How can one accurately calculate dosage—which presumes uniform distribution—when variation and unpredictability of concentration are the rules in these neoplasms? Worthy of note is the observation that in 32 cases normal thyroid tissue concentrated the isotope, whereas carcinoma tissue in the same radioautographic tissue section showed no evidence of concentration. We have seen no case in which carcinoma tissue concentrated more or even as much radioiodine as did normal thyroid tissue. This selective affinity for normal thyroid follicles would seem to be in keeping with the observation that concentration in metastatic lesions appears to increase after thyroidectomy or destruction of the gland (10).

Significance of the radioautographic demonstration of I^{131}

Very pertinent would be the question as to the amount of I^{131} necessarily present in thyroid tissue to give discernible darkening in the radioautograph. Marinelli and Hill have calculated the number of microcuries of I^{131} per gram of normal thyroid tissue which must be present to give positive radioautographs of a photographic density of 0.6 in a histologic section 10 microns thick. Their figures were 3.1 and 0.34 microcuries per gram of normal thyroid tissue for lantern slide and X-ray film emulsions respectively, presuming uniform distribution. These authors suggest that their calculated values might possibly need downward revision. Our finding of 21 cases of thyroid carcinoma, in which histologic sections gave no greater radioactivity counts than background by Geiger-Müller counters (method of Marinelli and Hill) but gave evidence of focal concentration of I^{131} in radioautographs, supports this view.

Direct measurement of the radioactivity of the histologic sections of the 21 cases showed that less than 0.2×10^{-3} microcurie of I^{131} per square centimeter was present. Assuming the histologic sections to average 10 microns in thickness (and thereby giving a volume of a cubic millimeter) the calculated amount of I^{131} present in the thyroid tissues must have been less than 0.2 microcuries per gram, presuming uniform distribution of the isotope.

However, the focal concentration of the isotope that was visible by radioautography may have been much higher than this value.

From our studies, it is obvious that distribution is not uniform and we believe that this fact explains, in part, why so many radioautographs demonstrated the isotope when Geiger-Müller counters did not. For a very small focus of radioactivity of the size of a thyroid follicle, easily visible in the radioautograph when viewed through the microscope, might not give rise to significant increase in radioactivity counts when a large area of tissue is counted against background activity. Possibly also a factor is the ability of the human eye to recognize differences in photographic density below a level of 0.6, which Marinelli and Hill used for their calculations.

Since our recent radioautographs were made by a method whereby tissue sections are mounted permanently on photographic emulsions and each is subjected to the histologic processes of de-paraffinizing, dehydration and staining procedures, as well as to exposure, and photographic development and fixation, it is manifest that many possibilities for artefacts, variations, and all sorts of undesirable effects exist. Because of these factors we do not believe that we can apply microdensitometric analysis to our radioautographic material at present. However, there is no theoretical reason why precise and accurate measurements of the amount of photographic darkening—and thereby a reasonable calculation of the amount of I^{131} present—cannot be accomplished if proper standardization of techniques can be effected. Such advances should reasonably be expected. An instrument has already been devised which should make microdensitometry of radioautographs relatively simple once they are processed with uniformity (11).

Marinelli and Hill have also calculated the amount of radiation received by tissues concentrating I^{131}. One microcurie of I^{131} destroyed per gram of tissue exposes the latter to 144 equivalent roentgens. Since the calculated lower levels of concentration of radioiodine necessary to give radioautographs are 3.1 and 0.34 microcuries of I^{131} per gram of normal thyroid tissue for lantern slide and X-ray film emulsions, respectively, this would mean an "average" dose of 450 and 49 equivalent roentgens. If the isotope is concentrated in the colloid (presumed to comprise one-eighth of the thyroid volume), "maximal" tissue doses of 3600 and 390 equivalent roentgens, respectively, would be received by the thyroid. Since positive radioautographs were obtained with concentrations less than 0.2 microcuries per gram of thyroid tissue the "average" dose in these cases would be less than 29 and the "maximum" dose less than 230 equivalent roentgens. From the standpoint of radiation protection it would be desirable to work with lower concentrations. Our limited data suggest that much lower "tracer" doses than previously used might be employed without sacrifice of radioautographic information.

Until microdensitometric analysis of radioautographs is practical, we cannot use radioautographic densities as a guide in estimating accurately tissues doses.

The question of the equivalent roentgen value of beta rays in terms of comparable X-rays and their respective biologic effects is beyond the scope of both the paper and the authors, but we have noticed in 1 case with metastatic alveolar and follicular carcinoma no significant destruction of tumor in a lesion whose estimated "average" dose received over a period of several courses of treatment was 500,000 equivalent roentgens. Radioautography demonstrated abundant concentration of isotope in the tumor.

Follicular function

The variation in concentration of isotopic iodine from follicle to follicle raises the question as to a possible phasic variation in the intrafollicular metabolism of iodine. Possibly follicles have rhythmic periods of activity and quiescence in regard to the concentration of iodine. One wonders whether a heavy uptake of radioiodine indicates a previous state of iodine deficiency or whether the follicle merely functions at a high level of activity and thereby requires more iodine. Comparison of the radioactive iodine in relation to the total iodine of the follicle would be helpful in the solution of this problem. The technique of historadiography (12) (13) as applied by Marinelli and Ferlazzo (14) to the thyroid gland enables one to judge, in relative terms, the amount of iodine present in thyroid follicles without destruction of the tissue section. We have been able, in a few cases, to make a radioautograph of radioactive thyroid tissue, remove the tissue section from the radioautograph, and place the same tissue section on another emulsion and make a historadiograph (15). Thus we might speak, in relative terms, of the amount of metabolic iodine (determined by radioautography) and total iodine (determined by historadiography) of a single follicle. However, the technique of historadiography shows only atomic iodine and other studies might have to be made simultaneously to determine whether the iodine shown to be present by historadiography was composed of iodide, diiodotyrosine, thyroxine or possibly other compounds.

SUMMARY

1. One hundred consecutive cases of carcinoma of the thyroid from patients who had received "tracer" or "therapeutic" doses of radioactive I^{131} were studied by the radioautographic technique. Forty-six gave radioautographic evidence of concentration of the isotope in the carcinoma.

2. The histologic pattern of the tumor appeared to have a significant association with the concentration of the isotope, in that types with abundant colloid were more likely to concentrate I^{131} than those types not containing colloid. The latter rarely concentrated the isotope.

3. Factors other than the presence of colloid must also be very significant, for some follicles showed no activity, whereas morphologically similar, neighboring follicles were heavily laden with I^{131}.

4. Positive radioautographs have been obtained showing concentration of I^{131} when the tissue concentration, as measured over a square centimeter, has been less than 0.2 microcuries per gram of tissue.

5. In no case have we seen a concentration of I^{131} in thyroid carcinoma tissue greater than, or equal to, the concentration in adjacent thyroid tissue. There were 32 cases in which a radioautograph contained both normal thyroid and carcinoma tissue; the former showed concentration of the isotope and the carcinoma gave no evidence of such concentration.

6. The uneven, variable concentration of radioactive iodine in normal as well as neoplastic thyroid tissue raises fundamental questions, such as the possible phasic function of the thyroid follicle. It also emphasizes the problem of the calculation of therapeutic doses. The rationale of radioactive iodine therapy must cope with this apparent inherent obstacle of the phasic nature of follicular function.

7. The total amount of iodine present in a thyroid follicle might possibly be a potent factor in determining whether radioactive iodine is concentrated in the follicle. It is hoped that the technique of historadiography, which reveals atomic iodine, in combination with radioautography may add pertinent information.

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Dr. Albert Keston, New York University School of Medicine, and Dr. S. M. Seidlin, Montefiore Hospital, New York City, kindly furnished the tissue and slides of 10 cases.

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A METHOD FOR THE PREOPERATIVE ESTIMATION OF FUNCTION IN THYROID TUMORS: ITS SIGNIFICANCE IN DIAGNOSIS AND TREATMENT*†

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MUCH has been learned about the relative function of thyroid nodules (1) by giving radioactive iodine (I^{131}) to patients with nodular goiter and making radioautographs from the excised tissue. With this method we have shown that some nodules have little or no function whereas, on the basis of unit area, others are more active than the extra-nodular thyroid tissue. The term "hyperfunctioning nodule or adenoma" has been used by some to imply the presence of clinical thyrotoxicosis (2, 3). New information revealed by radioautographs shows that nodules with excessive function may occur in patients *without* as well as in patients *with* thyrotoxicosis (1); therefore, the terms "hyperfunctioning nodule with thyrotoxicosis" and "hyperfunctioning nodule without thyrotoxicosis" have become necessary (1, 4).

A satisfactory method of estimating the relative function of thyroid nodules before their removal is of value in determining their nature. It assists in the anticipation of possible malignancy. It indicates the presence of a hyperfunctioning nodule even before the onset of overt thyrotoxicosis. It assists in differentiating between thyrotoxicosis resulting from a hyperfunctioning nodule on the one hand, and the diffuse hyperplasia of Graves' disease with an incidental nodule on the other.

Since the radioautograph has demonstrated that the uptake of radioactive iodine (I^{131}) can be used as an index of the biologic activity of thyroid tissue, we have attempted to measure the local concentration of radioactive iodine in thyroid nodules before their surgical removal.

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METHOD

The location of a source of radiation may be determined by using a Geiger-Müller counter and finding the point of maximal radiation by approximating it to that source. However, in the case of thyroid tissue which is buried deep in the neck, two features make distinction between intensities of point sources difficult: 1) The nodule concerned lies within a mass of thyroid tissue which itself contains radioiodine. 2) The overlying tissue of the neck makes it impossible to bring the counter sufficiently near the sources so that differences in the amounts of radiation coming from small areas in close proximity may be distinguished from each other. Therefore, a directional counter has been constructed and this has been found capable of localizing and differentiating relative concentrations of radioactive iodine over small areas of the thyroid.

This directional counter utilizes the principle of shielding a Geiger-Müller counter with lead through which a small portal has been made (Fig. 1, A and B). The following physical principles have been taken into consideration:

1. Sufficient lead must be interposed between the source and the counter in order to exclude all radiation other than that entering by way of the portal.

2. Since the intensity of radiation varies inversely as the square of the distance, it is necessary to have the counter as near the source as possible. Therefore, the lead shield must be of minimal thickness, compatible with No. 1.

3. There must be sufficient thickness of the lead on the other hand so that the pathway through the portal will permit only approximately parallel rays to enter the counter.

4. The diameter of the portal must be limited so that a relatively small area is surveyed and the principle of parallelism applied.

5. The portal, however, must be sufficiently large to admit adequate radiation for reliable counting, since only very small tracer doses of radioactive iodine are given to the patient.

Numerous observations were made with varying apertures, lead shielding and Geiger-Müller counters, until significant differences could be detected between the nodules and the extra-nodular tissue in patients who

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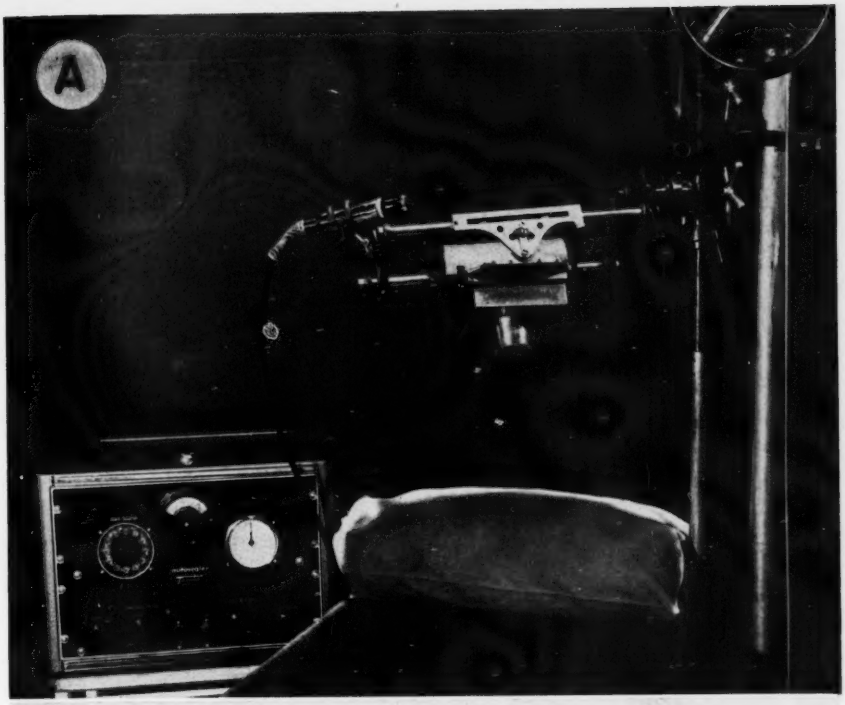
FIG. 1. A. *Directional counter apparatus.*

B. *Construction of directional counter represented diagrammatically.*

The apparatus consists of a platinum screen wall Geiger-Müller counter shielded with lead, through which a small portal has been made. The portal is placed directly over a given area of the thyroid and held rigidly in contact with the skin of the neck.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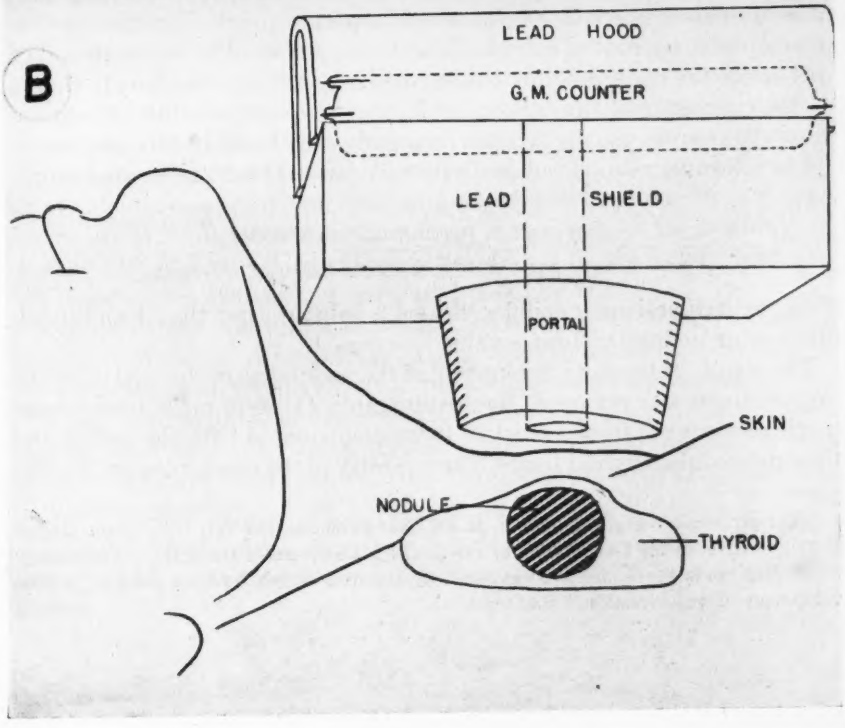
B
LEAD HOOD
G. M. COUNTER
LEAD
SHIELD
PORTAL
SKIN
NODULE
THYROID

had received only tracer doses of radioactive iodine. In the process of developing the technic, the efficiency of the gamma ray counter was raised considerably by replacing the copper screen cathode, with one of platinum.[1] The pattern of lead shielding appears in Figure 1 (A and B). The portal was 19 mm. in diameter through lead, of which the maximal thickness was 95 mm. The Geiger-Müller tube was fastened behind this shield and covered by a removable hood of lead measuring 15 mm. in thickness. The entire apparatus was suspended by an x-ray stand over the neck of the recumbent patient. The various adjustments on the x-ray stand made it possible to place the portal directly over any area of the thyroid and hold the apparatus rigidly in the desired position. In the more recent observations, a given area was first counted; then, without moving the apparatus or the patient, a lead plug accurately fitting the entire portal was inserted, in order to obtain background values. The net counts per minute over various areas of the thyroid were thus obtained by subtracting the background values.

After administering a tracer dose of radioactive iodine to a patient, forty-eight hours were allowed to elapse before counting over the thyroid, so that most of the circulating iodine would become fixed in the thyroid or be excreted.

Since December 1946, observations have been made on 41 patients with either a single discrete nodule, or several separate nodules arranged so that an acceptable amount of extra-nodular tissue remained to be counted. The area beside the trachea, if no nodules could be felt there, was usually chosen for the estimation of the radioactive iodine in the extra-nodular tissue, because the thyroid usually reaches its greatest thickness in this location.

The following ratio of radioactivity was derived from the in vivo counting:

$$\frac{\text{Net counts per minute of nodule}}{\text{Net counts per minute of extra-nodular thyroid}}$$

Thus, hyperfunctioning nodules yielded a value greater than 1 and hypofunctioning nodules yielded a value less than 1.

The results of the in vivo counting of the nodules were checked after the thyroid tissue was removed. Radioautographs (1) were made from microscopic sections cut from a block of tissue composed of both the nodule and the extranodular thyroid tissue. The intensity of the blackening on the film

[1] The authors are grateful to Prof. R. D. Evans and Marvin Van Dilla of the Radioactivity Center of the Department of Physics at Massachusetts Institute of Technology for having made these improved gamma ray counters which we have used in the development of our directional counter.

thus gave a measure of the intensity of the radiation in each type of tissue. The degree of blackening was measured with a microphotometer (5). The background haziness on the film was also determined and related to the above readings in the following fashion:

$$\frac{\text{Log of background} - \text{Log of nodule}}{\text{Log of background} - \text{Log of extra-nodular tissue}} = \text{The ratio of intensity of radiation in the nodule to that of the extra-nodular thyroid tissue.}$$

Thus, hyperfunctioning nodules yielded a value greater than 1, whereas hypofunctioning nodules yielded a value of less than 1.[2]

RESULTS

Table 1 shows the nodular vs. the extra-nodular ratios of radioactive iodine uptake as determined by the in vivo counting technic in column #6 and by the radioautographic technic in column #7. The 41 cases in Table 1 were arranged in order of diminishing ratio of activities of the nodules, based on the radioautographic method.

In the instances where the radioautographs clearly indicated that a nodule was hyperfunctioning, the preoperative in vivo observations in these patients proved reliable (Table 1, Cases 2 through 7). Most nodules with activity approximately equal to the extra-nodular thyroid were similarly identified by the in vivo counting technic (Table 1, Cases 8 through 17). With definite hypofunctioning nodules, the in vivo estimations were usually indicative of a relatively poor radioactive iodine uptake; however, the ratio derived from the in vivo counting was usually not as low as the actual ratio derived from the radioautographs. When counting over very inactive nodules a considerable amount of activity was often detected due to the extra-nodular tissue lying in front of and behind the nodule. This accounts for the failure to obtain ratios as low as they should be with hypofunctioning nodules (*see* lower part of Table 1).

Certain explanations may be offered for some of the discrepancies appearing in Table 1. A knowledge of the factors contributing to some of these has led to an improvement in the application of the technic.

A very careful physical examination of a nodular thyroid was of fundamental importance. The topographical relationships must be clear in order to apply the directional counter. The following are factors which may lead to misinterpretation:

[2] Care was taken to obtain a sufficiently large section of extra-nodular thyroid tissue so that possible impaired function resulting from compression by the nodule could be avoided.

TABLE 1. NODULAR VS. EXTRA-NODULAR RATIOS OF RADIOACTIVE IODINE UPTAKE

No.	Case identification	Multiple or single	Size in mm.	Necrosis	In vivo counting ratio of activity (*see* text)	Autoradiograph—ratio of blackening (*see* text)	Comments
1	38-48	M	30	Extensive	.395	65.3	* See text
2	81-47	S	28	Minimal	2.79	32.0	
3	61-48	M	30	Minimal	3.19	19.1	One nodule studied
4	5-47	M	33	Minimal	2.22	16.6	Nodules side by side; counted together
			16	0		(19.4)	
5	96-47	S	24	Minimal	4.25	8.14	
6	19-48	S	35	Minimal; fibrotic	4.60	6.40	
7	31-48	S	21	Minimal; cystic	2.17	3.15	
8	106-47	S	30×45	Partly cystic	1.69	1.89	
9	4-49	S	30	Fibrotic center	.850	1.617	*
10	43-48	M	30	Mostly cystic	.727	1.115	* Second nodule insignificant
11	45-48	S	25	Largely cystic	.699	1.11	*
					1.22 (4 mos. before)		
12	47-48	S	35	Minimal; cystic	.995	1.09	
13	40-48	S	20	Minimal	.939	1.08	
14	15-49	S	35×15	0	1.47	.926	
15	2-47	S	26	Minimal	1.15	.885	
16	15-48	S	19	0	1.88	.821	†
17	6-47	S	51	0	.95	.529	
18	11-47	M	11	—	.43	.272	Two nodules, well separated
			24	Largely cystic	.33	0	
19	35-48	S	11	Slightly cystic	1.17	.118	‡
20	7-47	M	25	0	.57	.0802	Nodules clustered
			12	0			
			12	0			
21	10-49	S	20		1.38	.0677	Graves' disease
22	13-47	S	30	Minimal	.59	.0662	
23	41-48	M	35	Partly cystic	.508	.057	Nodules side by side; counted together
			19	0		(.326)	
24	9-47	M	40	0	.29	.0416	Second nodule insignificant
25	15-47	S	146×94	Mostly cystic	.44	.0390	
26	32-47	S	53×36	Minimal	.47	.0336	
27	3-47	S	94×55	0	.35	.0217	Follicular adenocarcinoma
28	16-47	S	29	0	.90	.0197	Graves' disease
29	5-49	S	58	Largely necrotic	.538	.0187	
30	13-48	S	60×44	—	.611	.0086	
31	55-48	S	55		.862	.0072	†
32	87-47	M	60	0	.551	.0069	Papillary adenocarcinoma
33	58-48	M	50	Minimal	.750	.0060	Second nodule in opposite lobe insignificant
34	24-47	S	20	Entirely necrotic	12.8	0	†
35	52-48	S	12	0	1.64	0	‡
36	18-47	S	12	Entirely cystic	1.08	0	‡
37	13-49	M	29	0	.502	0	Second nodule, in opposite lobe, insignificant
38	97-47	S	78×55	Largely cystic	.43	0	
39	29-48	S	46	0	.43	0	
40	19-47	S	77×42	0	.21	0	Papillary adenocarcinoma
41	16-48	S	45	Moderate	.13	0	Small cell carcinoma encapsulated

The total absence of activity as shown by the radioautographs in the cases at the bottom of the table is not adequately reflected by the in vivo counting. Radiation is contributed by the functioning tissue in the immediate neighborhood of the inactive nodule. The method, however, is sufficiently accurate in most cases to indicate the presence of a hypofunctioning nodule.

Symbols Indicating the Probable Source of Individual Errors:

* = Necrosis of most of the nodule, resulting in marked reduction in the average uptake per gram as compared to the viable cells next to the capsule.

† = Unexplained cause of error.

‡ = Small size of the nodule, making the total mass relatively small compared to relatively larger amounts of extranodular thyroid in front of and behind the nodule.

1. *Irregularity in the gland:* Over some areas of the thyroid there is greater depth of tissue than in other areas. The thyroid tissue is thickest on each side of the trachea at the level of the isthmus and is often thinner over the tapering superior poles and isthmus.

2. *Position of the nodule:* If the nodule was in an area where the thyroid was rather thin, then the nodule made up a relatively larger per cent of the total tissue in the area counted, thus favoring an accurate estimate of the nodule. On the other hand, considerable normal thyroid tissue might be superimposed over the nodule as in the case of a nodule lying posteriorly beside the trachea. Under such circumstances the superimposed tissue might contribute in a major way to the activity detected, which could be falsely interpreted as coming from the nodule.

3. *Multiplicity of nodules:* In a case where multiple nodules were lying in close proximity to one another, individual nodules were sometimes difficult to demarcate on physical examination and thus impossible to localize under the portal of the directional counter. Other nodules, unnoticed on physical examination, can lie deep to the one under observation. In glands with multiple nodules, an area might have been chosen which was thought to be free of nodules; however, at operation this area sometimes proved to have other small nodules present. Because of this inability to estimate the function of nodules in diffusely nodular goiters such patients have been excluded from this report.

4. *Necrosis:* Necrosis is very common in hyperfunctioning nodules (1). The following observations on a patient serve to illustrate how necrosis in a nodule is one of the most important causes of error in in vivo counting:

The patient was a woman of 69 who had had nervousness, tremor of the hands, loss in weight, dyspnea and tachycardia for two years; and sweating, intolerance to heat and an increase in appetite for one year. Her basal metabolic rate was plus 67 per cent. A diagnosis of thyrotoxicosis was made. The patient's symptoms, according to her own estimate, had begun to abate before treatment was started. In fourteen days the symptoms had disappeared and the basal metabolic rate had reached 0. Although she had received 300 mg. of propylthiouracil daily for fourteen days, this decline was a much more rapid response than might be expected in a nodular goiter with this degree of hyperthyroidism. A tracer dose of radioiodine was given after the propylthiouracil had been stopped. Figure 2 illustrates how in vivo counting revealed much less radioiodine in the region of the nodule than in the extra-nodular thyroid tissue. When the nodule was removed it was found to be cystic and filled with liquid and debris. From the microscopic tissue section and its radioautograph (Fig. 3, A and B) it was evident that although the center had been necrotic, there was a rind of very active thyroid tissue just within the capsule and this had concen-

trated radioiodine far in excess of the extra-nodular tissue (Table 1, Case 1). Had this nodule been composed throughout of tissue comparable to that found just beneath the capsule, as it probably had been in the recent past, the in vivo counting of this nodule would probably have revealed

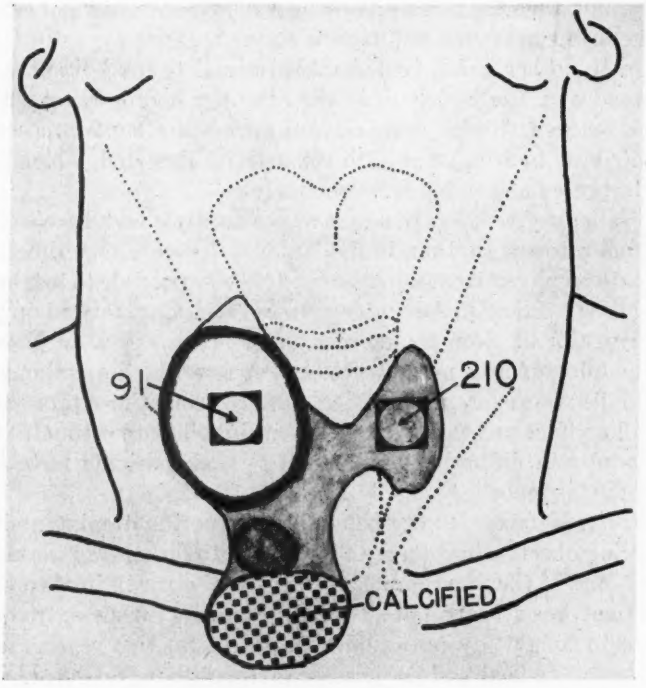

FIG. 2. *The results of in vivo counting over an extensively necrotic hyperfunctioning nodule.*
The numbers represent the net counts obtained by in vivo counting over the areas indicated. The shading illustrates the degree of blackening on the radioautograph made from the thyroid after removal.

A rind of very active tissue is noted just within the capsule of an otherwise necrotic nodule, patient's upper right. In vivo counting gave no clue to the presence of this hyperfunctioning tissue (Table 1, Case 1; and Fig. 3).

hyperactivity. From the in vivo observations, apparently made after necrosis had developed, there was no suspicion that the nodule contained hyperfunctioning tissue.

Realizing the high incidence of necrosis in hyperfunctioning nodules (1), we have been prompted in the case of larger lesions to make a tangential survey through the nodule. This method measures the radiation in a larger

proportion of the rind, rather than in the center, which may be necrotic.

In contrast to the above patient is another (illustrated in Figure 4; and Table 1, Case 5) who had a hyperfunctioning nodule which contained no necrosis. Here a cross-sectional estimate through the center of the nodule compared to the extra-nodular tissue left no doubt that this was a hyper-

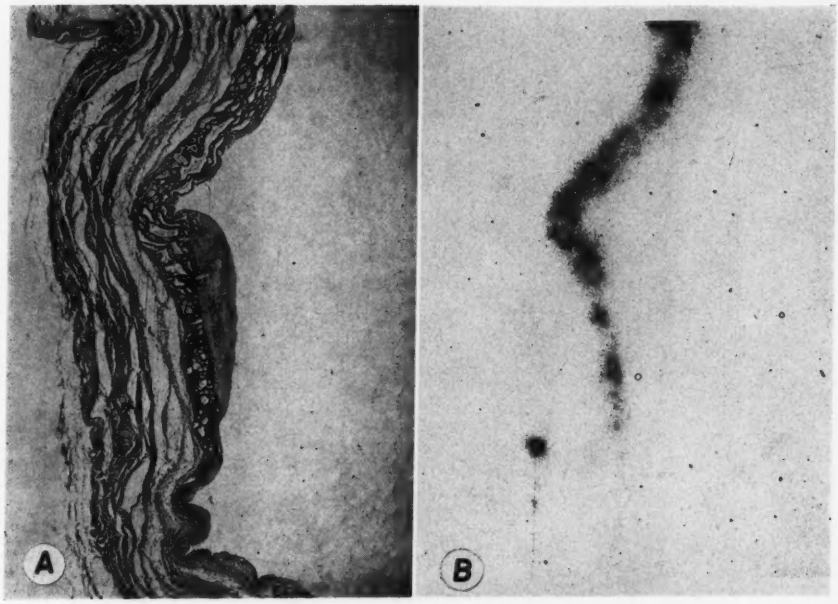

FIG. 3. A. *A microscopic section containing nodular and extra-nodular tissue.*

B. *Radioautograph made from the same section* (same tissue as in Figure 2, patient's upper right).

Note intense concentration of radioiodine in the remaining viable tissue just within the capsule (Table 1, Case 1).

functioning nodule, even though the patient had a basal metabolic rate of only plus 4 per cent.

5. *Small nodules:* It will be clearly seen in Table 1 that many of the errors were made in attempting to assay the function of nodules which were quite small (less than 1.5 cm. in diameter). Among these small nodules the detection of definite hyperfunction was usually much more reliable than the detection of hypo- or absent function. If there was a considerable depth of extra-nodular thyroid about an inactive nodule, the normal tissue could contribute sufficient radiation to obscure the absence of radiation in the nodule.

DISCUSSION

The foregoing observations show that the application of this method of study is of value in clarifying some of the diagnostic problems of nodular goiter.

The hyperfunctioning nodule: If a nodule is found to have relatively more uptake of radioiodine than the extra-nodular thyroid tissue, it may be assumed that the nodule is relatively hyperfunctioning. Such nodules are recognized as a source of clinical or masked hyperthyroidism which may produce cardiac damage insidiously. Hyperfunctioning nodules should be considered a potential cause of thyrotoxicosis (1) even though the toxic symptoms are not evident. The recognition and removal of this type of nodule is often followed by remarkable improvement in the patient's sense of wellbeing. The directional counter has been found useful in recognizing this type of lesion.

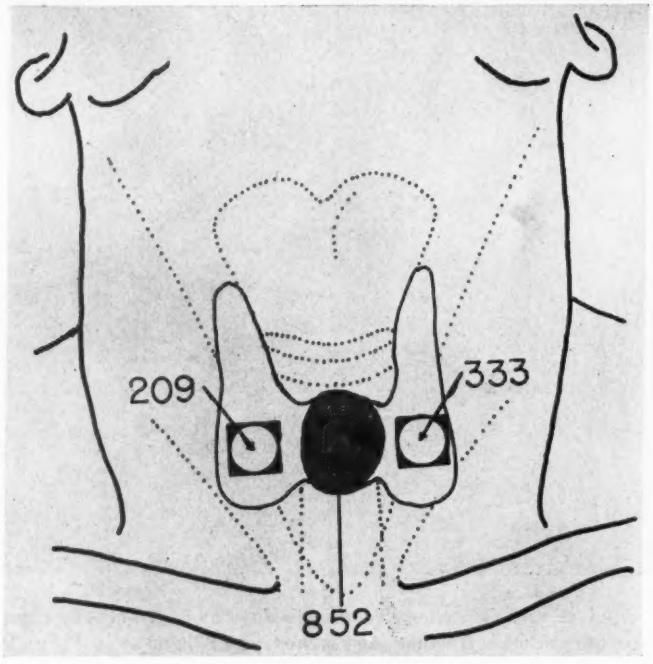

FIG. 4. *A hyperfunctioning nodule without necrosis.*

The numbers represent the net counts obtained by in vivo counting over the areas indicated. The shading illustrates the degree of blackening on the radioautograph made from the thyroid after removal.

Preoperative diagnosis was hyperfunctioning nodule without thyrotoxicosis (Table 1, Case 5).

Carcinoma: Two important facts stand out in our experience with carcinoma: 1. Carcinoma of the thyroid takes up less radioiodine than normal thyroid tissue (6, 7). 2. Eighteen per cent of patients with single nodules who are operated on are found to have a carcinoma (8, 9). Therefore, if a single nodule is found to have distinctly less function than the extranodular tissue this will exclude the hyperfunctioning group and increase

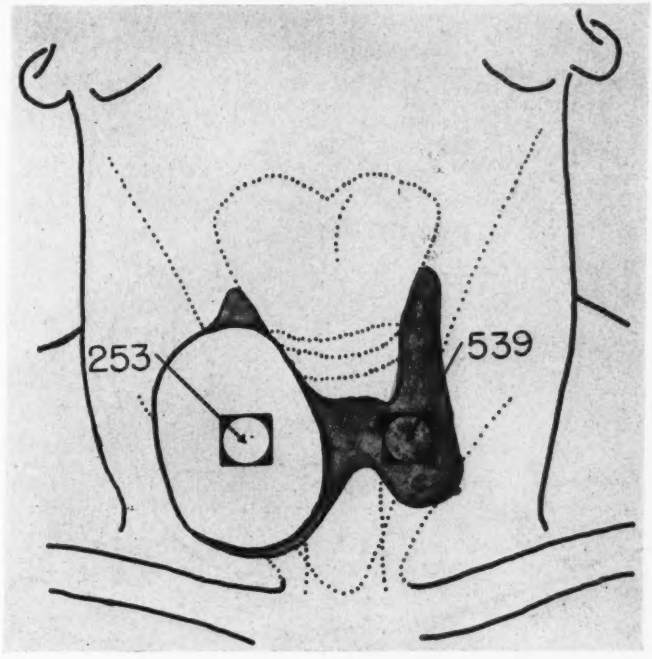

FIG. 5. *A benign hypofunctioning nodule.*
The numbers represent the net counts obtained by in vivo counting over the areas indicated. The shading illustrates the degree of blackening on the radioautograph made from the thyroid after removal (Table 1, Case 26).

(above 18 per cent) our suspicion of malignancy. Thus the preoperative finding of a distinctly hypofunctioning nodule makes the surgeon more wary in his approach to the lesion. Figure 5 represents the results of in vivo counting over a single nodule which histologically was benign and radioautographically possessed minimal function (Table 1, Case 26). Figure 6 represents a somewhat larger nodule which was proven to have no radioiodine and was carcinoma (Table 2, Case 40).

Graves' disease with an incidental nodule vs. a hyperfunctioning nodule with thyrotoxicosis: In many patients with thyrotoxicosis and a nodular goiter it is difficult to be sure whether the increased metabolism is due to a

diffuse hyperplastic gland, irrespective of the nodules which may be present, or due to a hyperfunctioning nodule. We know from our experience and that of others that if a nodule is functioning sufficiently to produce thyrotoxicosis there will be suppression of the remaining thyroid (1, 3, 10, 11, 12). In such a case the activity as measured by the directional counter will be concentrated in the area of the nodule (Table 1, Cases 2 through 7).

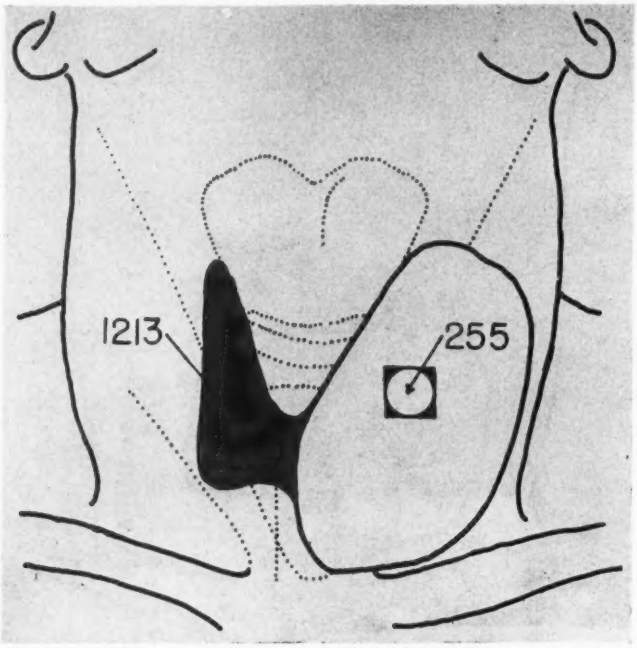

FIG. 6. *A malignant nodule without function.*

The numbers represent the net counts obtained by in vivo counting over the areas indicated. The shading illustrates the degree of blackening on the radioautograph made from the thyroid tissue after removal.

The absence of function in this large solitary nodule was indicated by the negligible counts found over it in contrast to the extra-nodular tissue (Table 1, Case 40).

On the other hand, in Graves' disease the radioactivity will be fairly uniformly distributed throughout the gland. The fact that the nodule may not be functioning, however, may be concealed by the radioactivity in the hyperplastic gland lying in front of and behind the nodule in question. Suffice it to say that if the patient has definite thyrotoxicosis but no evidence of localized activity in the nodule, then the diagnosis is Graves' disease (Table 1, Cases 21 and 28). Figure 7 illustrates the results of in vivo

counting in a patient with Graves' disease. The radiation was of approximately uniform distribution all over the gland, thus indicating that the well encapsulated discrete nodule was not excessively active. The radioautograph, as described by the shading of the gland (Fig. 7), showed practically no radioiodine uptake in this nodule.

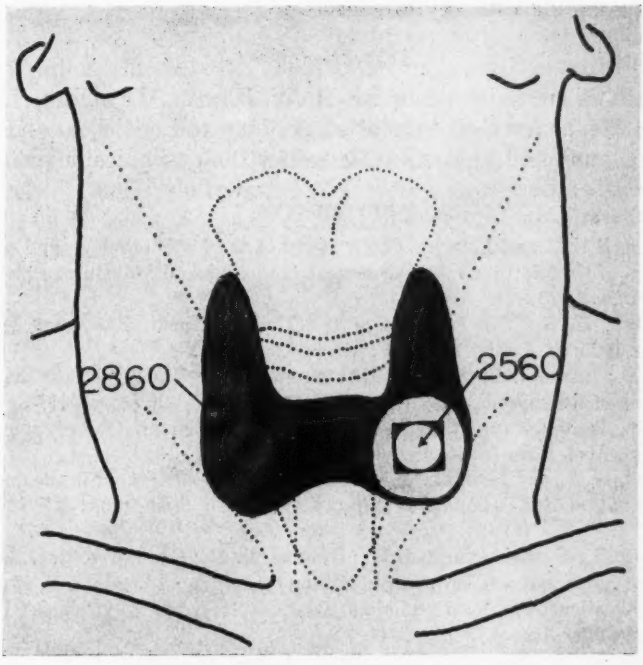

FIG. 7. *Graves' disease with an incidental nonfunctioning nodule.*

The numbers represent the net counts obtained by in vivo counting over the areas indicated. The shading illustrates the degree of blackening on the radioautograph made from the thyroid tissue after removal.

There was an excessive uptake of radioiodine throughout the hyperplastic gland. Had the nodule been the source of the thyrotoxicosis, it would have shown a high concentration while that of the remaining atrophic tissue would have been minimal (Table 1, Case 28). Compare with Figure 4.

SUMMARY

Radiation from radioactive iodine has been used for estimating preoperatively the relative function of thyroid nodules. The accuracy of the method has been measured by radioautographs made from the tissue removed at operation. Results of observations on 41 cases of nodular goiter have been

analyzed. Reasons for certain errors are discussed. This method is of value:

1) In raising the suspicion of carcinoma.

2) In the diagnosis of hyperfunctioning nodules with or without thyrotosicosis, and

3) In distinguishing between the diffuse hyperfunctioning gland of Graves' disease with an incidental nonfunctioning nodule, and a hyperfunctioning nodule with thyrotoxicosis.

Acknowledgment

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A SIMPLIFIED METHOD FOR THE DETERMINATION OF THE PROTEIN-BOUND BLOOD IODINE AND ITS CLINICAL APPLICATION*†

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FOR a number of years the blood iodine has proved a valuable adjunct in evaluating the disturbed activity of the patient's thyroid gland. This is especially true since the advent of protein-bound blood iodine determinations (1, 2). Despite its clinical significance, however, its use has been largely limited to the research laboratory owing to difficulties inherent within the various methods. As a consequence we have attempted to utilize and apply an extensive simplification in devising a method which would permit of more widespread use of blood iodine determinations. This method is simplified, chemically accurate, and has shown good clinical correlations.

Protein-bound blood iodine determinations are particularly helpful in indicating when hypermetabolism or hypometabolism is not of thyroid origin. Conversely there may be hyperthyroidism or hypothyroidism which is not associated with corresponding deviations in the basal metabolic rate. In these, the protein-bound blood iodine[1] becomes a valuable aid in arriving at the clinical diagnosis. In addition, blood iodine determinations are of value in following the different types of thyroid patients through their indicated therapy. We have employed them extensively for this purpose.

In the past, determinations of the blood iodine have been highly specialized procedures requiring a great degree of skill and training (3, 4, 5, 6, 7). However, they have been simplified to a degree, with the more recent adoption of the use of ceric sulfate (8–15). In addition, the procedure has been largely limited to research laboratories because of required space, special equipment and precautions against iodine contamination.

In developing this simplified method for the clinical laboratory we have employed only those articles usually available, and presumably in use or readily obtainable by the majority of laboratories. These consisted of an

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[1] Hereafter referred to as the PBI.

Evelyn colorimeter, a size 2 International centrifuge, a constant temperature water bath, electric hot plates, a small stream of compressed air, standard chemicals and a hood. The only exception to this was a special, easily constructed, glass iodine analysis assembly (Fig. 1). In addition to the use

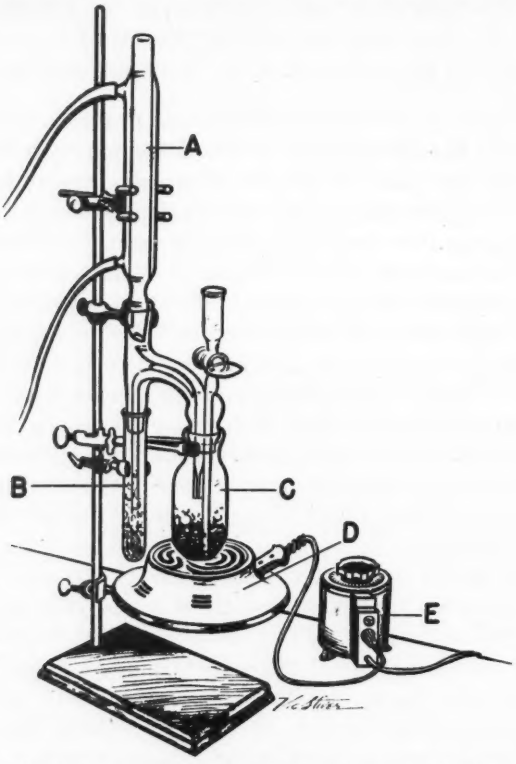

FIG. 1. *Distillation apparatus for the determination of the blood iodine in the clinical laboratory*

The central part of the apparatus is all of one piece. It has ground glass joints to receive a reflux condenser (A), a colorimeter tube (B), and a reagent flask (C). The hot plate (D) may be accurately controlled by a powerstat (E).

of the above equipment only five feet of laboratory desk working space is required.

A laboratory technician can be taught the method in a short period of time; however, it requires previous chemical experience for one to be able to set up the method for the first time. Even this may be greatly simplified if one orders the necessary solutions made up to specification and uses them as stock solutions, since they are all stable. Since the method is essentially a

closed procedure no special precaution need be taken against contamination beyond that of washing and rinsing the glassware.

A single competent technician can complete an analysis in less than three hours and can do about ten in an eight-hour day.

The details of the method have been presented elsewhere (16).

Briefly, a 9 ml. specimen of blood is drawn from the patient, usually immediately after determining the BMR, under basal conditions. This is transferred to a Wassermann tube and allowed to clot. The clot is packed down by centrifugation. A 3 ml. specimen of serum is then pipetted volumetrically into the special 250 ml. reagent flask (Fig. 1, C). Somogyi reagents are added which precipitate the proteins (17, 18, 19). The flask is then centrifuged and the supernatant fluid, which contains the soluble iodine, decanted off and placed in a colorimeter tube.

The protein precipitate remaining in the 250 ml. centrifuge flask contains the protein-bound, physiologically significant fraction of the blood iodine. This precipitate is washed once with water and then oxidized with a mixture of chromic and sulfuric acids (3–10). The 250 ml. centrifuge flask is then attached to the distillation assembly (Fig. 1, C) and brought to a boil. Phosphorous acid (4) is added through a reagent tube and the iodine is released. This iodine is carried by steam through a delivery tube to the potassium hydroxide trap which is a colorimeter tube ground at the top so that it may be attached to the distillation assembly (Fig. 1, B).

After ten minutes' distillation the colorimeter tube is detached. Ceric sulfate and arsenious acid are added. The rate of the reaction between ceric sulfate and arsenious acid is determined by the blanching of the yellow color of the ceric sulfate. This rate of reaction is directly proportionate to the amount of iodide present (8–15). Absolute values of samples are determined by direct comparison with previously prepared standard curves. The colorimeter tube containing the soluble fraction is treated in the same manner.

In the laboratory this method was found to have an average error of plus or minus five per cent, so that it could be said to be accurate chemically. The method then was given a clinical trial.

From September 1, 1948 to May 1, 1949 over 800 analyses were accomplished. Several were from other departments in the University Hospital, many from other hospitals. In our further discussion, the clinical diagnosis, the protein-bound blood iodine and the basal metabolic rate will be considered.

We are presenting only our own patients and only those who stated they have taken no form of iodine or antithyroid drug. There were 87 of these. Due to the selection which takes place before patients come to our clinic we had a large number already receiving some form of iodine or antithyroid drug. Consequently, we had many cases unavailable for this study. Also due to this selection, a large number of patients were referred to our clinic because it was suspected that their basal metabolic rate was not a true index of their thyroid function. Therefore we find an unusually high disagreement between the basal metabolic rate and the clinical diagnosis.

We have found the normal range of the protein-bound serum iodine, using our method, to lie between 3.7 and 6.7 micrograms per cent. The average normal is 4.6 micrograms per cent.

In this series of 87 patients the basal metabolic rate was in disagreement with the clinical diagnosis in 20, whereas the blood iodine disagreed with the clinical diagnosis in 8 cases. Of the 20 patients in whom the basal metabolic rate was in disagreement, 6 had an anxiety state, 3 had disturbance in their respiratory physiology, 2 had infections and 2 presented "masked" hyperthyroidism: in the remaining 7 the findings are not clear. In 2 of the 7, the clinical diagnosis was in question and the patients did not return for further study. In addition the blood iodine was in agreement with the basal metabolic rate in these 2, whose tentative diagnosis was probably in error. This leaves 5 out of the 87 in whom there is no known reason why the basal metabolic rate disagreed with the clinical diagnosis, or a percentage of 5.7.

Of the 8 patients in whom the blood iodine disagreed with the clinical diagnosis, 2 had a questionable tentative diagnosis as discussed above. To these must be added a third case of questionable tentative diagnosis because of incomplete study. Another patient had obviously had iodine recently, but claimed to be taking no medication. This leaves 4 unaccounted. Of these, 1 was a hyperthyroid individual with a borderline normal value of 6.7 micrograms per cent. The other was hypothyroid with a borderline normal value of 3.7 micrograms per cent.

Thus there are but 2 out of the 87 in whom there is no known reason why the protein-bound blood iodine disagreed with the clinical diagnosis, or a percentage of 2.3.

SUMMARY

A simplified, rather rapid method for determining the protein-bound blood iodine is described. It has been found chemically accurate and yields results which have a good clinical correlation.

Acknowledgment

We express our thanks to June Swanson, B.S., Robert Lieberman, B.A. and David Ucker, B.A. for their valuable technical assistance.

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BASAL METABOLISM TESTING UNDER PENTOTHAL ANESTHESIA*

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THE test for basal metabolism is accepted as a valuable aid in clinical diagnosis. Although an elevated basal metabolic rate suggests overactivity of the thyroid gland, it is recognized that it may occur in many other clinical states unrelated to the thyroid gland. Interpretation of an increased metabolic rate, therefore, demands careful evaluation of the entire clinical picture.

Hyperthyroidism resulting from primary hyperthyroidism (exophthalmic goiter) or adenomatous goiter is the most common cause of an elevated metabolic rate, and the administration of large doses of desiccated thyroid has the same effect. Fear of the metabolic test, neurocirculatory asthenia, various emotional states, and fever frequently cause an increased metabolic rate. Less common causes include dyspnea resulting from heart failure, endocrine diseases such as acromegaly (at times associated with an overactive adenomatous goiter), and Cushing's syndrome, blood diseases such as leukemia and polycythemia, pregnancy, pain, discomfort and muscle activity such as occurs in spasmodic torticollis or Parkinson's disease. A few rarer conditions such as alcoholism, hypertension, pheochromocytoma, aortic stenosis and coarctation of the aorta must also be considered in evaluating a reported elevation of the metabolic rate.

It is, of course, essential that the metabolic test be carried out under proper conditions so that the result obtained represents the metabolism in a truly basal state. All extraneous nervous factors should be eliminated so that a state of true physical and nervous relaxation is obtained. In most patients this is accomplished by having them rest in a quiet room for thirty minutes before proceeding with the metabolic test. Before hospital accommodations became so difficult to obtain, our patients were usually kept in the hospital overnight and the metabolic test was taken the following morning in the resting state.

Since in certain nervous patients routine rest is occasionally insufficient to accomplish a basal state, various measures must be employed. These include serial testing so as to acquaint the patient with the test, administration of phenobarbital ($\frac{1}{2}$ grain three times a day) for three to four days before the test, or the administration of nembutal (pentobarbital sodium),

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1½ grains, the night before and again one hour before the test in the morning. In most instances, by following one of these measures a satisfactory basal metabolic rate is obtained, but even with these precautions one still encounters a few individuals who require greater sedation. An ideal basal state would be normal sleep, but sleep induced voluntarily obviously is impossible to procure. It was thought feasible, therefore, to induce sleep by the intravenous administration of pentothal (sodium ethyl thiobarbiturate), which acts rapidly and is readily controlled, in an effort to obtain the desired basal state for testing.

METHOD OF STUDY

The patient to be studied is taken to the metabolism room in a fasting state, having received a hypodermic injection of 1/150 grain of atropine sulfate one hour before. Two consecutive determinations of the metabolic rate are then taken in the usual way. A trained anesthetist sprays the throat with 10 per cent cocaine and induces anesthesia by the intravenous injection of pentothal, administering the least amount of drug necessary to produce complete relaxation. If the breathing is free and easy, the regular metabolism rubber mouthpiece is inserted. If the breathing is not free, a metal airway attached to the rubber mouthpiece is inserted to facilitate adequate air exchange. Adhesive tape is then tightly applied around the lips to prevent any possible air leak. Two consecutive metabolic tests are then taken. During the test the chin is supported to facilitate normal breathing and a small amount of additional pentothal is injected when necessary to produce uniform relaxation.

PATIENTS STUDIED

A total of 45 patients (Table 1) were studied by this plan of metabolic testing. The 18 patients initially tested had normal routine basal metabolic

TABLE 1

Clinical condition	No. patients tested
Normal subjects	18
Hyperthyroidism	13
Hypertension	4
Vasomotor instability	6
Pheochromocytoma	1
Aortic stenosis (adenomatous goiter)	1
Aortic insufficiency (rheumatic heart disease), anxiety state	1
Spasmodic torticollis (Parkinson's disease)	1
Total	45

rates and no evidence of thyroid disease. Thirteen patients having various degrees of unquestionable hyperthyroidism due to primary hyperthyroidism or adenomatous goiter with hyperthyroidism were tested. Initially, hyperthyroid patients were studied to determine the effect of the anesthesia on the metabolic rate and more recently the test has been used as an aid in determining the true basal metabolism in hyperthyroid patients who have associated nervous states making it difficult to take a satisfactory test. Most of these latter patients were receiving antithyroid treatment in preparation for thyroidectomy and confirmation of an euthyroid state was desired prior to proceeding with thyroidectomy. The other cases studied consisted of patients with hypertension and associated tension states, vasomotor instability with associated nervous manifestations, aortic stenosis, aortic insufficiency with associated anxiety state and spasmodic torticollis. All of the patients tested, other than the normal subjects had elevated metabolic rates when the test was taken in the routine way.

RESULTS

Eighteen patients who had normal basal metabolic rates and no evidence of metabolic disease were used as control subjects. The average metabolic rate of these patients was plus 4 per cent (Table 2). The average metabolic

TABLE 2. AVERAGE METABOLIC RATES*

Number of patients studied (normals)	Average metabolic rate before anesthesia, per cent	Average metabolic rate under pentothal, per cent
17	+4 (Range, +16 to −10)	−9 (Range, +9 to −26)

* In each patient two to three determinations of the basal metabolic rate were taken before, and two to three after anesthesia. The average of these rates was used in calculating the average for the group.

rate under pentothal anesthesia was minus 9 per cent, a drop of 13 in the metabolic percentage. The amount of pentothal required to produce light anesthesia varied from 0.5 Gm. to 1.2 Gm., an average of 0.9 Gm. This drop of 13 per cent in the metabolic rate obtained under anesthesia compares favorably with that reported (1) to follow normal sleep, when the decrease is from 7 to 12 per cent.

Patients with severe hyperthyroidism showed little change in the metabolic rate under anesthesia. One patient (Fig. 1), aged 65 years, with hyperthyroidism due to an adenomatous goiter, had uniformly high metabolic rates, averaging plus 62 per cent. Under pentothal anesthesia the average rate was plus 60 per cent. A second patient with hyperthyroidism due to a

recurrent adenomatous goiter had two readings of the metabolic rates of plus 50 per cent before anesthesia; under anesthesia the rates were plus 46 and plus 40, or a decrease of 7 in the metabolic percentage. The amount of pentothal required to induce anesthesia in these hyperthyroid patients was

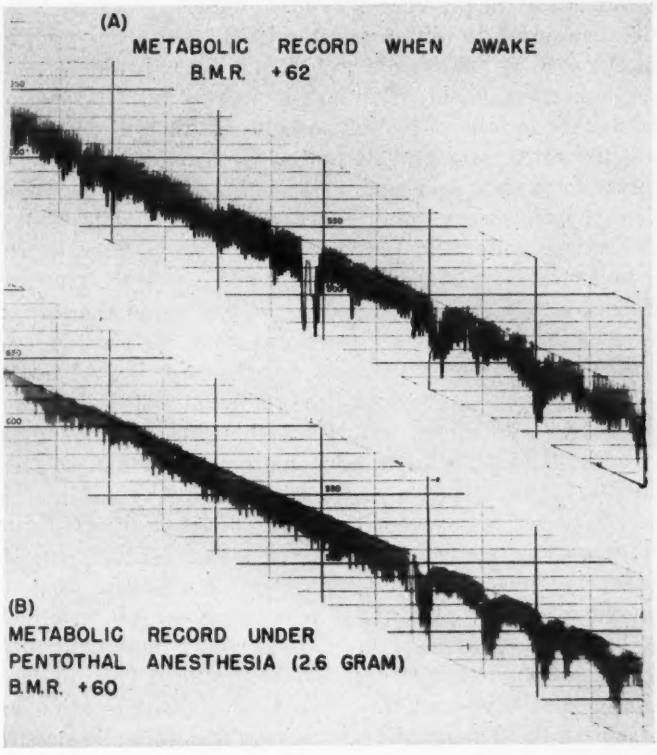

FIG. 1. Metabolic record before and during anesthesia, of a patient with severe hyperthyroidism.

found to be considerable—2.6 Gm. in the first patient and 2 Gm. in the second patient.

The other hyperthyroid patients studied were also found to have sustained hypermetabolism under anesthesia, unless there was associated neurocirculatory instability making the routine basal metabolic rate unreliable. One very unstable hyperthyroid patient with an initial basal metabolic rate of plus 54 per cent received 300 mg. of propylthiouracil daily for 153 days, an amount which is sufficient to produce an euthyroid state in

most patients with a similar metabolic rate. A basal metabolic rate at the end of this time was plus 66 and the next day plus 53 per cent. She seemed clinically improved but it was difficult to determine whether the high metabolism was due to residual hyperthyroidism or just to inherent nervousness. The basal metabolic rates under anesthesia were plus 34 and plus 30 per cent, indicating the presence of residual hyperthyroidism. The amount of pentothal required to obtain anesthesia, 2.5 Gm., was additional evidence for the presence of hyperthyroidism. A second similar unstable patient with hyperthyroidism, after four months of antithyroid treatment, had basal metabolic rates of plus 65 and plus 60 per cent. Under pentothal anesthesia the rates were plus 34 and plus 40 per cent, indicating continued hyperthyroidism, and making further antithyroid treatment necessary before thyroidectomy could be undertaken with safety. A third patient with a large goiter and anxiety neurosis received antithyroid treatment for four months with a gain of 21 pounds in weight. The initial basal metabolic rate was plus 68 per cent and at the end of therapy it was reported as plus 28 per cent. Under anesthesia the metabolic rate was still above normal, being plus 24 per cent. Further antithyroid treatment for two months was advised, at which time the basal metabolic rate was plus 7 per cent and thyroidectomy was done without reaction. A fourth patient with hyperthyroidism due to an adenomatous goiter had an initial basal metabolic rate of plus 30 per cent. She received antithyroid treatment with propylthiouracil for fifty-seven days and gained $5\frac{1}{2}$ pounds during this period. The basal metabolic rates at this time were plus 27, plus 34, plus 35 and the next day, plus 40 and plus 45 per cent. Under pentothal anesthesia the metabolic rates were plus 13 and plus 14 per cent; it was thought safe to proceed with thyroidectomy since the increased metabolic rate was attributed to an unsatisfactory metabolic test resulting from nervousness. A thorough subtotal thyroidectomy was done without reaction and three months later the basal metabolic rate was again abnormally elevated, being plus 51 per cent. A fifth hyperthyroid patient with an adenomatous goiter whose initial basal metabolic rate was plus 27 per cent received antithyroid treatment for sixty-one days. At the end of this time the rates were plus 40 and plus 35 per cent, although clinically she was greatly improved. The metabolic rates under anesthesia were plus or minus 0 and minus 15 per cent. Thyroidectomy was done without reaction. Other hyperthyroid patients with similar problems were studied and equally informative results were obtained, which aided in the determination of the readiness of the patients for safe thyroidectomy, our prerequisite for operation being an euthyroid state.

A metabolic test was taken under anesthesia on a hyperthyroid patient at the height of a thyroid storm which developed subsequent to an abdomi-

nal exploration for an acute abdominal condition. Previously, it was impossible to perform a test under such conditions. A metabolic rate of plus 100 per cent was recorded, indicating the very high rate of metabolism in this type of metabolic upheaval. Spinal anesthesia, a procedure suggested as a means of therapy for thyroid storm, did not lower the metabolic rate.

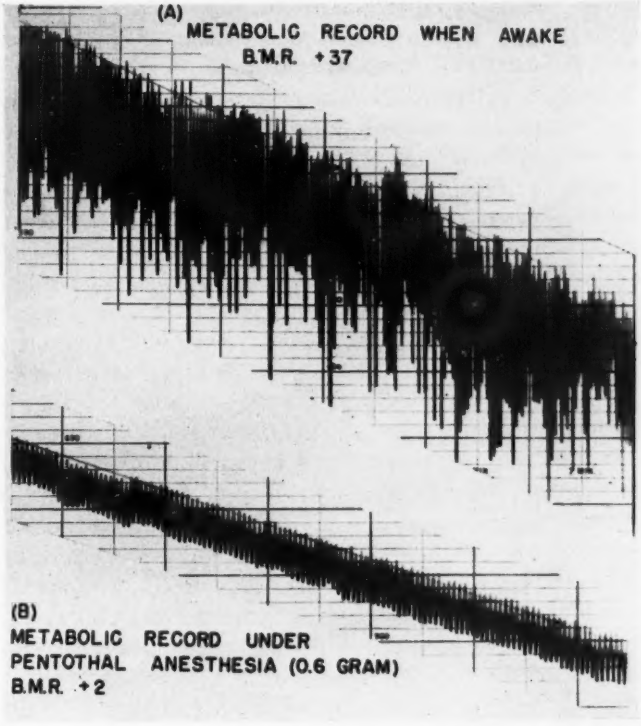

FIG. 2. Metabolic record before and during anesthesia, of a patient who presented suggestive symptoms of hyperthyroidism. Final diagnosis was profound psychoneurosis.

Metabolism testing which was carried out on patients whose metabolic rates were elevated and in whom the diagnosis of hyperthyroidism was in question was most helpful clinically. A woman of 32 years, who was first seen in May 1947, complained of weight loss, extreme nervousness, palpitation, irritability, insomnia and tremor of the arms. One and a half years previously she was thought to have hyperthyroidism; the basal metabolic rate was plus 29 per cent, and for one year she took iodine intermittently. On examination, the pulse was 110. There was a very coarse, jerky tremor

of the arms; there were no abnormal eye signs and the thyroid gland was normal to palpation. The basal metabolic rate was plus 45 per cent. She manifested many signs of nervous instability and although the diagnosis of hyperthyroidism seemed unlikely, it was thought advisable that she have a therapeutic trial of antithyroid drugs. Therefore, propylthiouracil was

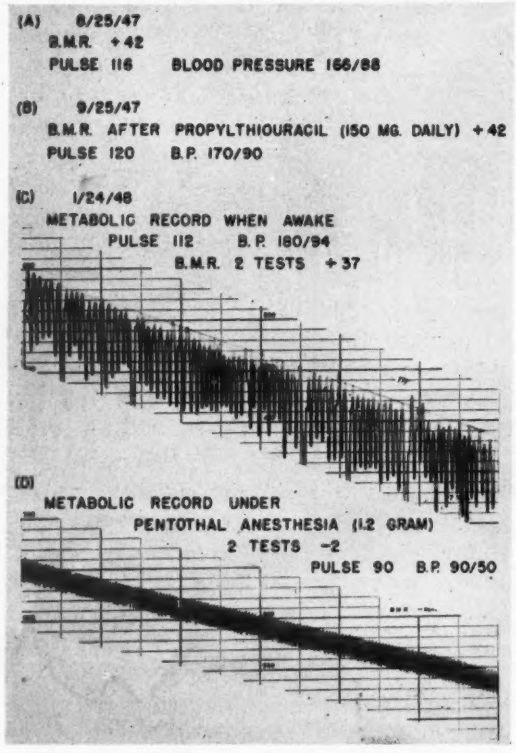

FIG. 3. Metabolic record before and during anesthesia, of a patient who was suspected of having hyperthyroidism but who failed to respond to antithyroid treatment. Final diagnosis was vasomotor instability.

given continuously in a daily dose of 200 to 300 mg. for the next six months. This medication did not produce the slightest subjective or objective benefit. Since she had difficulty taking the metabolic test, studies were carried out under anesthesia (Fig. 2). The preanesthesia rates averaged plus 37 per cent, the metabolic record showing evidence of a marked hyperpnea. Under anesthesia the metabolic rate fell to plus 2 per cent and the pulse

rate fell to 78. Only 0.6 Gm. of pentothal was necessary to produce satisfactory anesthesia. These results were interpreted as evidence to rule out the possibility of hyperthyroidism (spontaneous or factitious) and it was concluded that the problem was a psychogenic one.

A similar problem was that of a woman of 30 years (Fig. 3) who, since a miscarriage, had complained of irritability, choking sensation, sense of a lump in the throat, nervousness, emotionalism, palpitation, weight loss in spite of a good appetite, shakiness and intolerance to heat. For many years the pulse and blood pressure had been known to be fluctuant. A metabolic rate had been reported as plus 39 per cent. Physical examination revealed a pulse rate of 132 beats per minute; the blood pressure varied from 188 to 190 mm. systolic, and from 88 to 110 mm. diastolic. The heart was overactive and there was flushing of the face and neck. The thyroid gland seemed normal in size and consistency. There was a slight tremor of the hands, which were cold and moist. The metabolic rate was plus 42 per cent pulse 116, weight 86 pounds and blood pressure 166 mm. systolic and 88 mm. diastolic. Because the symptoms were suggestive of hyperthyroidism and since the metabolic rate was elevated, although no thyroid enlargement was felt, the patient was given a therapeutic trial of propylthiouracil. After one month of administration of propylthiouracil in a daily dose of 150 mg., the metabolic rate was still plus 42 per cent and pulse rate 120. Propylthiouracil was given for another month in a daily dose of 200 mg., without any subjective or objective improvement. Metabolic studies were then carried out under anesthesia. The preanesthetic rates averaged plus 37 per cent. Under anesthesia the metabolic rates were minus 2 per cent, the pulse was 90 and the blood pressure 90 mm. systolic and 60 mm. diastolic. The evident hyperpnea noted previously was altered to one with a more rapid rate but not as deep. These studies led to the conclusion that this patient's symptoms and increased metabolic rate were due to marked neurocirculatory instability.

A patient with severe spasmodic torticollis was suspected of having complicating hyperthyroidism, since she had lost considerable weight, was extremely nervous and had moderate tachycardia. The thyroid gland was not enlarged but hyperthyroidism was still considered. The metabolic rate was plus 67 per cent. Under pentothal anesthesia the jerking of the neck was relieved and a metabolic rate of plus 5 per cent was obtained. This drop in the rate to normal under anesthesia was interpreted as evidence to eliminate the possibility of hyperthyroidism.

Even in the very recent literature (2) a significant incidence of hypermetabolism was reported in patients with compensated hypertension. Our experience at the Lahey Clinic does not agree with this opinion since a truly elevated metabolic rate in a hypertensive patient without the

presence of hyperthyroidism is a rare observation. Should a hypertensive patient, irrespective of the height of the blood pressure, have a goiter and a maintained elevation of the basal metabolic rate, the presence of hyperthyroidism should be accepted. Should a hypertensive patient without goiter have an elevated basal metabolic rate, underlying nervousness should

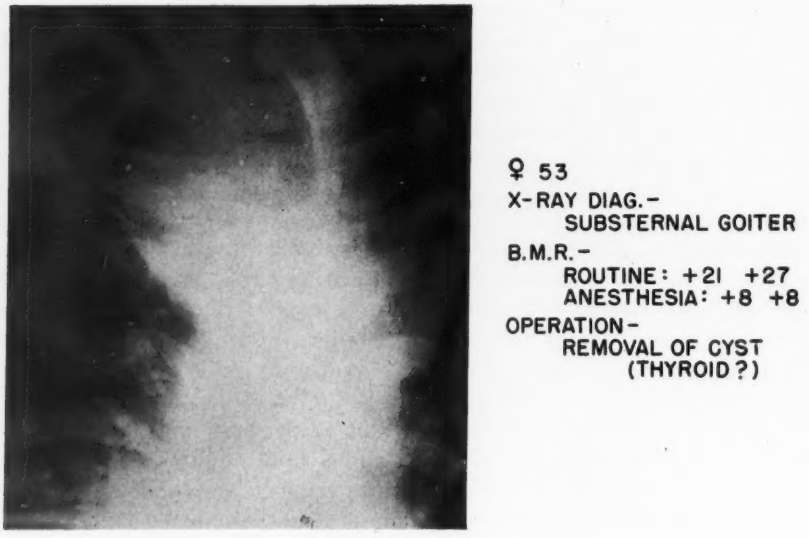

FIG. 4. Superior mediastinal tumor which had the characteristics of substernal goiter with slight tracheal deviation.

be considered as the reason for the increased rate since, with adequate sedation or anesthesia, a normal rate will be obtained. Four such hypertensive patients have been observed. The first patient had basal rates of plus 21 and plus 27 per cent, and under anesthesia plus 8 and plus 8 per cent. A second patient with basal rates of plus 30 and plus 22 per cent, under anesthesia had rates of plus 8 and plus 8 per cent. A third hypertensive patient had rates of plus 22 and plus 27 per cent and under anesthesia, minus 2 and plus 7 per cent. The fourth patient with an adenomatous goiter, without symptoms of clinical hyperthyroidism, had hypertension and basal metabolic rates of plus 47, plus 25 and plus 20 per cent. Under anesthesia the basal rates were plus 4 and minus 2 per cent. Thyroidectomy was done, with a satisfactory postoperative course. The drop in the metabolic rate under anesthesia, requiring little pentothal, seemed to indicate that the elevated preanesthesia rate of patients with hyperten-

sion is the result of a tension state and that hypertension *per se* is not a cause of hypermetabolism.

Another case in which metabolism studies of value were done under anesthesia, was that of a woman aged 39 years with hypertension. On roentgenologic examination of the chest (Fig. 4), a mass was demonstrated in the right superior mediastinum just below the clavicle. Although she showed no clinical signs of hyperthyroidism, the basal metabolic rates were plus 21 and plus 27 per cent even after sedation. The question then arose as to whether hyperthyroidism was present; if it were present, antithyroid treatment was indicated prior to thyroidectomy. As the patient was somewhat apprehensive, it was thought advisable to obtain further determinations of the basal metabolic rate under pentothal anesthesia. These rates were found to be plus 8 and plus 8 per cent, which was thought to rule out hyperthyroidism. Exploratory operation was done without delay and a cyst, 5 by 3 by 2 cm., was found which was thought to be related to the right lobe of the thyroid. The anesthesia and postoperative course were satisfactory, confirming the absence of hyperthyroidism. The elevated basal metabolic rate in this patient was interpreted as being due to nervousness and tension, perhaps related to the hypertension.

A patient 30 years of age, with known sustained hypermetabolism of twenty years' duration, who had had both thyroidectomy and x-ray treatment to the region of the thyroid without improvement, was found on study to have metabolic rates of plus 58, plus 60 and plus 60 per cent. Under anesthesia the rates were plus 62 and plus 53 per cent. Further studies revealed the presence of a pheochromocytoma which was successfully removed. Thirteen days after operation the basal metabolic rate was plus 8 per cent. In this case the sustained hypermetabolism, even under anesthesia, indicated that there was a definite cause for the high metabolic rate which required investigation.

Another interesting diagnostic problem was that of a patient with calcified aortic stenosis who also had a moderately large adenomatous goiter. Since repeated basal metabolic rates in this case were elevated (Fig. 5) and since he did not present evidence of heart failure to account for the maintained elevation, the possibility of hyperthyroidism had to be considered. Because the presence of hyperthyroidism could not be ruled out as the cause for the elevated metabolism, antithyroid treatment was given for eighty days. At the end of the time the basal metabolic rate was still elevated. Under anesthesia the rate was slightly reduced (plus 27 per cent) but it was sufficiently elevated to indicate hypermetabolism. In spite of the increased metabolic rate a subtotal thyroidectomy was done, without reaction. After operation, the basal metabolic rate continued to be elevated and when last taken four months after operation it was plus 27 per cent.

Elevated metabolism simulating hyperthyroidism has been reported (3) to occur in some patients with aortic stenosis but its cause remains unexplained. In the patient here reported the metabolic rate did not respond to antithyroid treatment and the rate remained elevated after a thorough thyroidectomy. This patient may represent an example of nonthyroid

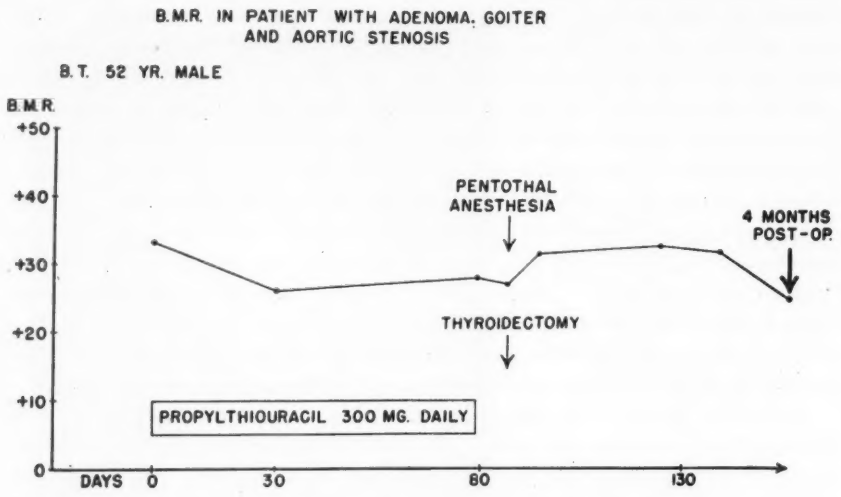

FIG. 5. Metabolic tests of patient with aortic stenosis and adenomatous goiter. Hypermetabolism continued after subtotal thyroidectomy.

hypermetabolism due to aortic stenosis. The basal metabolic rates of 6 other patients with calcified aortic stenosis were normal, indicating that only rarely does an elevated metabolic rate occur in this type of cardiac condition.

CONCLUSIONS

A reported elevated basal metabolic rate must not be accepted as certain proof of the presence of thyroid hyperactivity. Since other conditions may exist to elevate the metabolism, the entire clinical picture must be known in order to determine the significance of an elevated metabolic rate. Also, the metabolic rate is significant only if it is actually taken in a truly basal state. In some instances a basal state is difficult or impossible to obtain in spite of methods routinely used to eliminate extraneous factors such as exist in some nervous patients.

Metabolic tests were taken under pentothal anesthesia in an attempt to secure a truly basal state. In normal patients the metabolic rate dropped an average of 13 per cent under pentothal anesthesia and an average of

1 Gm. of pentothal was necessary to induce anesthesia. Patients with hyperthyroidism showed little drop in the metabolic rate under anesthesia and 2 Gm. or more of pentothal was required to produce sleep. Patients with greatly elevated metabolic rates due to various nervous states had a drop in the basal metabolic rate to normal under anesthesia, indicating an absence of hyperthyroidism. This method of metabolism testing induces a perfect basal state—the resulting rate being void of all nervous and muscular factors. In the occasional problem patient with an elevated metabolic rate this procedure may be used to good advantage; such cases are reported in this paper. The test has been used to advantage in determining the safest time for operation on patients with hyperthyroidism who have associated nervous states and in whom satisfactory metabolism tests are difficult to obtain. Hypertensive patients with elevated rates had a lowering of the metabolic rate under anesthesia, showing that the occasional increased rate of the hypertensive patient is related to a tension state and not to the hypertension.

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A STATISTICAL STUDY OF THE CLINICAL SIGNIFICANCE OF LYMPHOCYTIC AND FIBROCYTIC REPLACEMENTS IN THE HYPERPLASTIC THYROID GLAND*†

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RELATIVELY little is known concerning the significance of lymphocytic and fibrocytic replacement in the hyperplastic thyroid gland (1). Thyroiditis has been studied in detail as an independent clinical and pathologic entity (2–4); the association of thyroiditis and exophthalmic goiter in the same patient, however, has received scant attention (5–8). It has been assumed that lymphocytic replacement and fibrocytic replacement of the hyperplastic parenchyma are related in some way to reduction in the intensity of the disease but their relationship to specific clinical signs and symptoms has not been extensively investigated (9).

One year before Parry established exophthalmic goiter as a definite clinical entity in 1825, Conradi (10, 11) distinguished between inflammation of the goitrous gland, the so-called strumitis of later writers, and inflammation of the previously normal gland. Between 1825 and 1914 lymphocytes and fibrous tissue in the hyperplastic thyroid gland attracted the attention of numerous workers, including Kocher (12), Berry (13), Lewis (14), MacCallum (15) and others (16, 17). It was not until Brünger's report (18) of 2 cases in 1914, however, that thyroiditis in exophthalmic goiter became recognized as a definite entity. Plummer (19) in 1923 reported 2 cases of exophthalmic goiter, in both of which chronic thyroiditis was found on microscopic examination of resected tissue. In both cases myxedema later developed. Searls and Bartlett (3) in 1926 presented their criteria for distinguishing between thyroiditis and thyroiditis in exophthalmic goiter. Smith, Pool and Olcott (20) in 1934 noted the similarity between the lymphoid tissue in exophthalmic goiter and that of struma lymphomatosa. Broders (21) in 1936 pointed out that varying degrees of fibrous and lymphocytic infiltration are present in all cases of exophthalmic

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† Abridgment of thesis submitted by Dr. Whitesell to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

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goiter. Hellwig (22) in 1939 concluded that histologic changes characteristic of so-called lymphadenoid goiter are occasionally found in thyroid glands resected because of exophthalmic goiter.

In the present study an attempt has been made to examine the effect of varying degrees of lymphocytic and fibrocytic replacement on the basal metabolic rate and on the incidence of auricular fibrillation, exophthalmos and postoperative myxedema.

Approximately 600 cases in which exophthalmic goiter was treated at the Mayo Clinic from 1923 through 1938 were reviewed. Eighty-six cases were finally selected from this larger group for study. It was necessary to exclude many cases because of inadequate preoperative and postoperative clinical data and because of insufficient information concerning the ultimate results after thyroidectomy. Other cases were excluded because of treatment of the thyroid which might have caused an increase in fibrosis or lymphocytosis, such as ligation of vessels, radiation therapy or injections of various agents into the thyroid. All cases of recurrent exophthalmic goiter were excluded for the same reason, as were cases in which degenerating adenomas were found in the resected tissue. Finally, definite thyroiditis had to be present to permit inclusion of the case; that is, cases of exophthalmic goiter uncomplicated by thyroiditis were excluded. The final group of 86 cases obviously was highly selected and cannot be considered as representative of all cases of exophthalmic goiter. Originally, an attempt was made to review consecutive cases but the effort was early abandoned because of lack of pertinent data.

As mentioned previously, not only the microscopic changes more or less characteristic of exophthalmic goiter, but also varying degrees of lymphocytic or fibrous thyroiditis were present in each gland studied. All patients had been treated with iodides before thyroidectomy (23). The type of thyroiditis in each gland was first classified as lymphocytic or mixed fibrolymphocytic (Fig. 1). In the former, parenchymal replacement predominantly was by lymphocytes with little accompanying fibrosis, whereas in the latter, definite regions of fibrosis were present in addition to lymphocytic replacement. In some cases lymphocytes were predominantly in small foci, in others lymphocytes were scattered diffusely and in still others definite lymph follicles were present. Fibrosis occurred as either localized or generalized increase of interlobular septa, as obviously parenchymal scarring or as penetration of fibrous tissue into the lobule between individual acini.

Six to eight blocks of tissue taken at random from each of 86 surgical specimens were sectioned and stained. In all, approximately 600 slides were prepared with six to eight slides on each case in the series. Serial microscopic fields were examined in such a manner as to cover each section

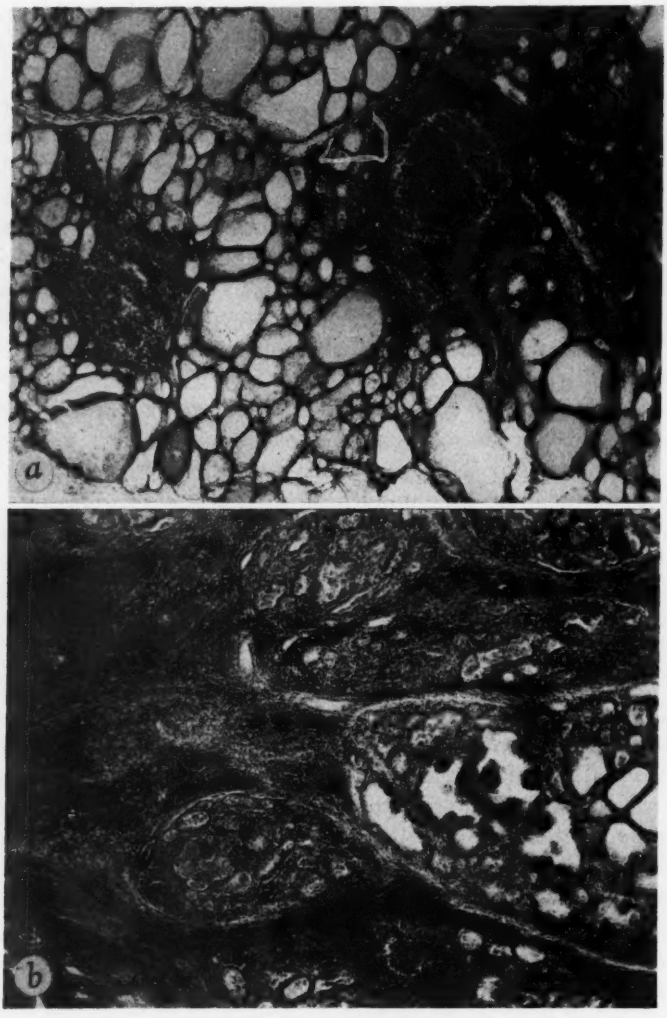

FIG. 1. Examples of: (*a*) the lymphocytic type of parenchymal replacement, and (*b*) the mixed fibrolymphocytic type (×55).

of tissue in its entirety. Each field was graded individually according to the extent of parenchyma replaced. Arbitrarily, grade 1 represented replacement from 0 to 25 per cent of the field; grade 2, 25 to 50 per cent of the field; grade 3, 50 to 75 per cent of the field, and grade 4, replacement exceeding 75 per cent of the field (Figs. 2 and 3). After approximately

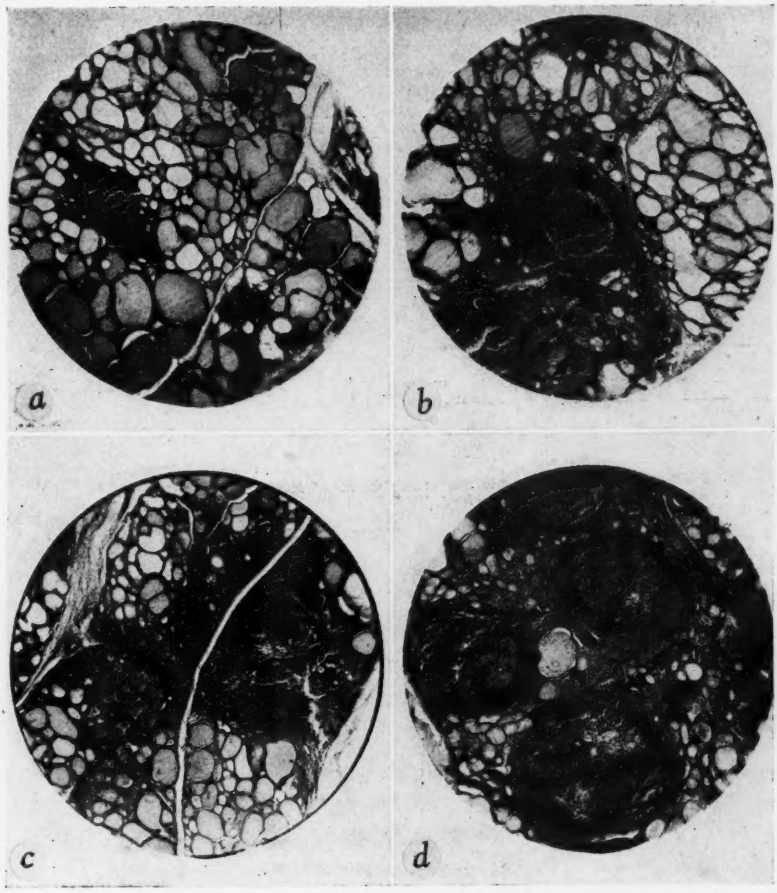

FIG. 2. Examples of grades 1 to 4 parenchymal replacement of the lymphocytic type: *a*. Grade 1, *b*. Grade 2, *c*. Grade 3, *d*. Grade 4 (×55).

150 fields had been graded in this manner for each gland, the mean grade and the standard deviation of the grades of parenchymal replacement were calculated.

The following case has been included as an illustration of the method. The patient was a woman aged 41 years with classic exophthalmic goiter of one year's duration. Subtotal thyroidectomy was carried out after preparation with iodides. The resected thyroid tissue weighed 60 Gm. The immediate postoperative convalescence was uneventful but myxedema developed subsequently. In our examination of the gland, 206 fields representing seven different regions from both of the lobes of the thyroid were

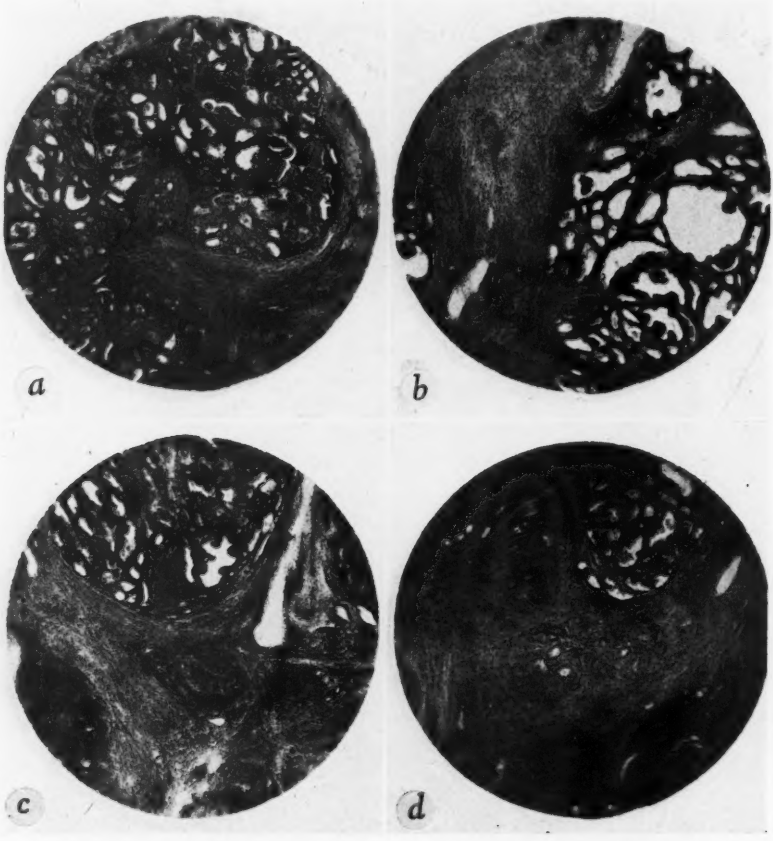

FIG. 3. Examples of grades 1 to 4 parenchymal replacement of the mixed fibrolymphocytic type: *a.* Grade 1, *b.* Grade 2, *c.* Grade 3, *d.* Grade 4 (×55).

graded as previously described. The findings are shown in detail in Table 1. The mean grade and its standard error were calculated in terms of the observed grade and these were converted to per cent by the appropriate conversion factors as shown in the table.[1]

Investigation of the clinical significance of parenchymal replacement by the procedure just described was adopted originally in an effort to find a

[1] In this case the standard deviation being 0.88 grade unit, in per cent it is 0.88 ×25 per cent = 22.0 per cent; the standard error of the determined mean is given by dividing this by the square root of 206, the number of observations. To make the similar translation for the mean, one must subtract 12.5 per cent after multiplying by the factor 25, since grade 1 represents 0 to 25 per cent, which is centered at 12.5 per cent.

TABLE 1. DISTRIBUTION OF FIELDS BY GRADE*

Slide	Number of fields					
	Total	Grade				
		0	1	2	3	4
1	37	4	25	6	2	0
2	29	0	3	4	22	0
3	21	0	11	8	2	0
4	27	0	3	7	17	0
5	29	0	12	9	8	0
6	27	1	13	8	5	0
7	36	0	5	16	15	0
Total	206	5	72	58	71	0

* Mean = 1.95 grade units = 36.3 per cent ± 2.5 per cent. Standard deviation = 0.88 grade unit = 22.0 per cent.

somewhat more precise method for evaluating the degree of thyroiditis than the one usually employed by the pathologist. It has long been recognized that myxedema does not uncommonly develop after thyroidectomy in those cases in which the thyroid parenchyma has been replaced extensively by lymphocytes and fibrous tissue. However, at least among our cases, there were curious instances in which myxedema failed to develop

TABLE 2. DISTRIBUTION OF GRADES

Grade*	Number	Per cent of cases	Standard deviation, (mean†)
Less than 0.60	10	11.6	0.51
0.60–0.79	21	24.4	0.65
0.80–0.99	16	18.6	0.69
1.00–1.19	12	14.0	0.80
1.20–1.39	7	8.1	0.72
1.40–1.59	6	7.0	0.88
1.60–1.79	2	2.3	1.04
1.80–1.99	4	4.7	0.98
2.00 and more	8	9.3	0.58
Total	86	100.0	

* Grade 1 represents 0 to 25 per cent replacement; grade 2, 25 to 50 per cent replacement; grade 3, 50 to 75 per cent; grade 4, more than 75 per cent.

† The mean of the standard deviations of each of the glands in the designated group.

in spite of a report of advanced thyroiditis in the surgical specimen; and conversely myxedema developed not uncommonly following reports of lesser degrees of thyroiditis.

The distribution of grade in the 86 cases examined and the variability of the grades in the individual glands as measured by the mean standard deviation are shown in Table 2. The size of the standard deviations, indi-

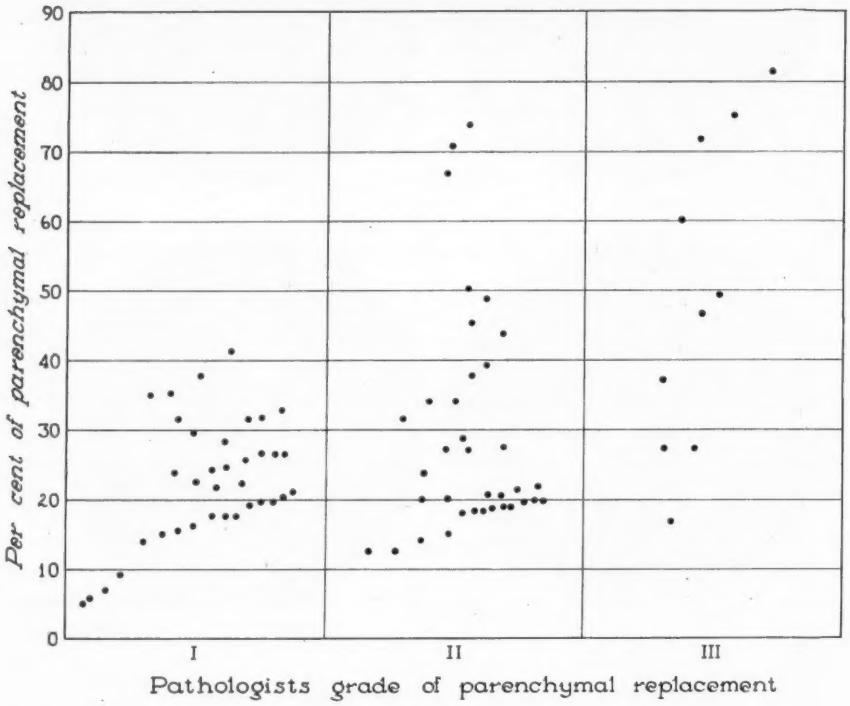

FIG. 4. A comparison of the pathologists' grades with the calculated percentage of parenchymal replacement.

cating considerable variability of the degree of replacement from region to region within any particular thyroid, probably explains the occasional discrepancies mentioned previously. To illustrate the differences between the pathologist's impression of the grade of thyroiditis obtained by viewing a portion of the gland and that resulting from a detailed examination of the entire gland, grade of replacement as judged by the pathologist was compared with that found in this study (Fig. 4). It should be pointed out

that the use of grades by the pathologist had no exact definition. For example, grade 1 was taken to mean slight thyroiditis whereas grade 4 was taken to indicate marked thyroiditis. It is apparent that the group considered by the pathologist to show lesser degrees of thyroiditis actually included cases showing rather advanced replacement when the entire gland was examined and graded, and that conversely, in the group considered by the pathologist to show extreme thyroiditis there were cases with lesser degrees of replacement. The estimated degree of thyroiditis may be fallacious therefore unless many sections from several regions in the thyroid are examined.

RESULTS

Since age and sex are important in the predisposition to exophthalmic goiter (24), both factors were examined in the present series of cases. The mean age of patients with the mixed fibrolymphocytic form of replacement was 5.4 years greater than that of patients with the lymphocytic type. Male patients uniformly showed 10 to 20 per cent less replacement than did female patients in corresponding decades. Unfortunately, we were unable to draw any conclusions concerning the influence of the duration of the disease on type or extent of parenchymal replacement, principally because of our inability to assign in many cases a definite date to the onset of the disease.

Three of the more significant objective findings encountered in cases of exophthalmic goiter were studied in an effort to judge the influence of lymphocytic and fibrocytic replacement on the clinical symptoms of this disease. Those selected were the basal metabolic rate, auricular fibrillation and exophthalmos, all of which lend themselves to objective appraisal.

It has been generally recognized that the basal metabolic rates are on the average lower in cases of exophthalmic goiter with accompanying thyroiditis than in cases of exophthalmic goiter without associated thyroiditis. Our findings were in accord with this generalization. In order to evaluate the basal rates accurately, only patients who had not received iodides for at least four weeks prior to testing were included in this phase of the study. The type of parenchymal replacement had little bearing on the basal rate. Thus, 39 patients with the lymphocytic form had a mean basal rate of plus 45.9 per cent whereas 29 patients with the fibrolymphocytic type had a mean rate of plus 43 per cent. The extent of parenchymal replacement, on the other hand, was more significant. As the extent of replacement increased, there was a definite trend toward a reduction in the rate. The basal rates within any particular grade of parenchymal replacement, however, showed a wide range of variability (Fig. 5).

Auricular fibrillation was present in this series in 9.3 per cent of cases. Although there was a somewhat higher incidence of the arrhythmia in the

mixed fibrolymphocytic form of replacement than in the lymphocytic form, extent of replacement was far more important than type. In all cases of fibrillation the thyroid glands exhibited replacement of less than 25 per cent. In general, an inverse relationship existed between the incidence of fibrillation and the proportion of the gland replaced.

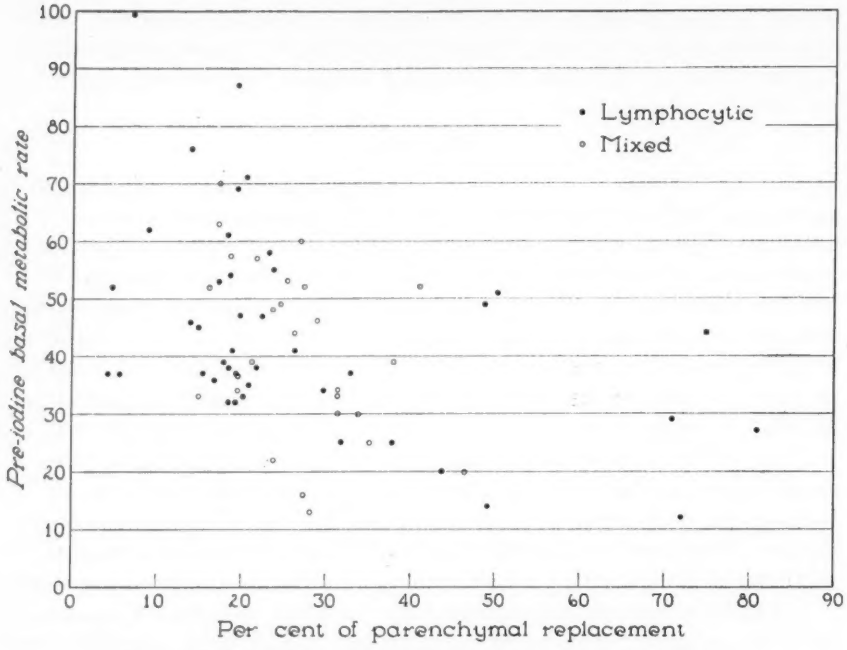

FIG. 5. Relationship of the basal metabolic rate to the mean percentages of parenchymal replacement.

Reported incidence of exophthalmos in exophthalmic goiter varies greatly (25, 26). In the present series of 86 cases, 38.4 per cent of patients were considered to have exophthalmos on clinical examination. Although the importance of the type of parenchymal replacement is reflected in an incidence of exophthalmos of 50 per cent in the fibrolymphocytic type as contrasted with 36 per cent in the lymphocytic type, the extent of replacement was again of far greater significance. When the incidence of exophthalmos was plotted against the proportion of parenchyma replaced, a smooth curve was obtained showing a definite increase in the incidence of exophthalmos with increasing degrees of replacement, at least up to 50 per cent replacement. Sixty per cent of the patients with replacement of this degree had exophthalmos (Fig. 6).

It is of interest in this connection that Mulvany (27) and Means (28, 29) have expressed the opinion that patients in whom an intensification of exophthalmos develops after thyroidectomy can be identified preoperatively by the mildness of their clinical findings. Similarly, when extensive replacement of parenchyma is found, the symptoms tend to be less severe than when little replacement is encountered, at least in terms of basal metabolic rate and the incidence of fibrillation.

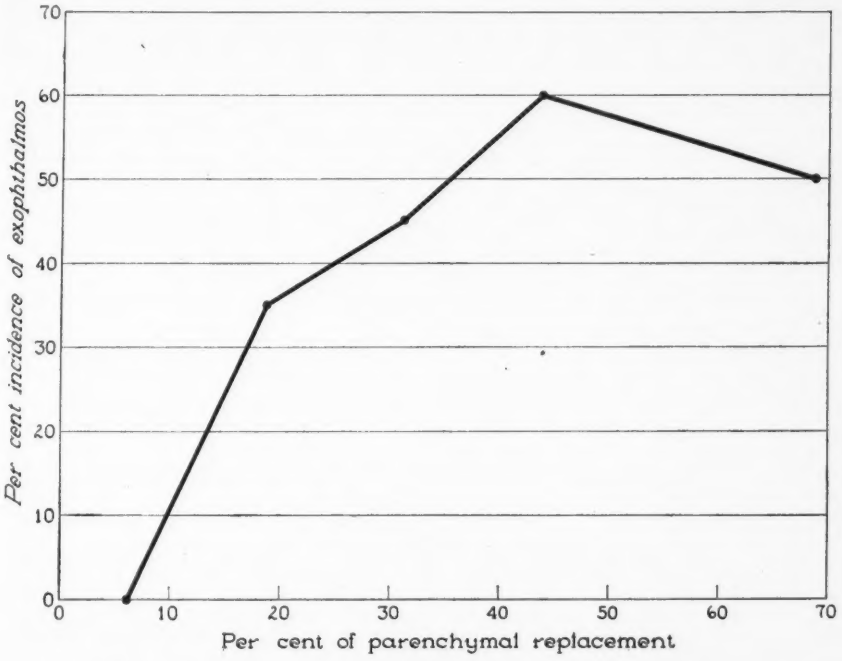

FIG. 6. Relationship of the incidence of exophthalmos to the mean percentages of parenchymal replacement.

Although lymphocytic and fibrocytic replacements in exophthalmic goiter are clinically important in many respects, from a surgical standpoint the direct relationship between such replacements and postoperative myxedema is far more significant. The work of Kocher in 1883 and the Report of the Clinical Society of London (30) in 1888 first directed attention to the relationship of thyroidectomy to myxedema. The inadequate function of thyroid tissue preserved after subtotal thyroidectomy, when myxedema develops, has been attributed to any or all of the following: removal of too much tissue, reduction of blood supply of the remnant,

prolonged aftertreatment with iodine and pathologic changes in the remnant (31–33). Though the exact factor or factors responsible still remain obscure, the present study afforded an opportunity to examine the last possibility.

The incidence of postoperative myxedema in this series of 86 cases with varying degrees of lymphocytic and fibrocytic replacement was 29 per cent. In 37 per cent of the patients with mixed fibrolymphocytic replace-

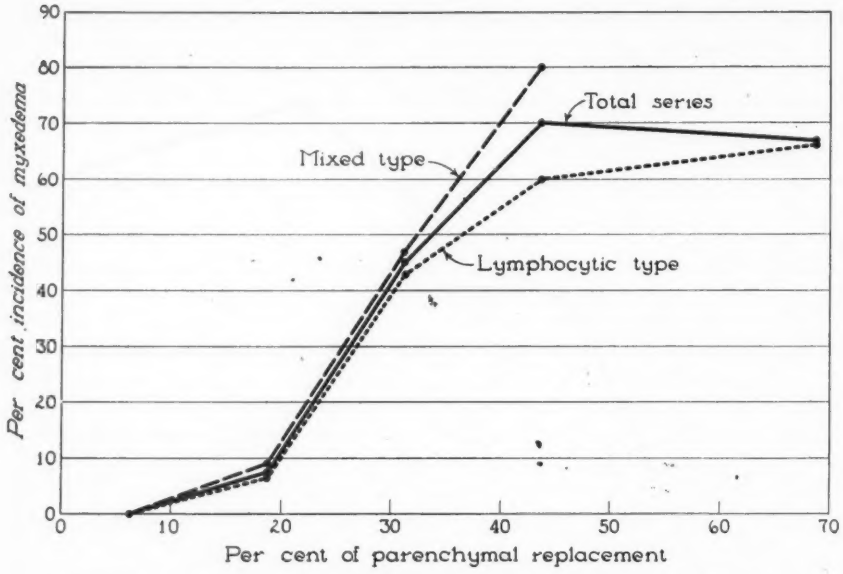

FIG. 7. Relationship of the incidence of postoperative myxedema to the mean percentages of parenchymal replacement.

ment, myxedema developed postoperatively, as compared to 24 per cent of the patients with the lymphocytic type. The greater tendency of myxedema to develop in patients with the mixed type of replacement can be explained on the basis of more marked replacement in general in cases with this type of replacement, suggesting that the thyroprivic effects of these cells are additive.

A positive correlation was found between the extent of lymphocytic and fibrocytic replacement and the incidence of myxedema following subtotal thyroidectomy (Fig. 7). When the incidence of myxedema was plotted against the proportion of parenchyma replaced, the incidence rose steeply to 70 per cent of cases when 40 to 50 per cent of the parenchyma had been replaced. A histologic evaluation of the degree of parenchymal re-

placement would seem therefore of considerable importance in predicting the development of postoperative myxedema. In all probability the amount of thyroid tissue necessary for adequate physiologic function varies with the extent of lymphocytic and fibrocytic replacement. Since the amount preserved is inadequate to prevent the development of myxedema in cases in which marked replacement has occurred, it is probable that in such cases greater amounts of parenchyma than usual should be preserved. We are somewhat reluctant to advise this, however, until more data become available and until the evaluation of the grade of replacement becomes more exact.

SUMMARY

Lymphocytes and fibrous tissue have long been recognized as integral parts of the pathologic picture of exophthalmic goiter but the significance of such tissue has not been widely studied. Previous assumptions, based on isolated cases, have linked increased replacement of parenchyma by lymphocytes and fibrous tissue to reduction in toxicity. In 86 cases of exophthalmic goiter with associated thyroiditis the mean grade and the standard deviation of the grade of parenchymal replacement by thyroiditic tissue were calculated after examination of multiple sections of tissue from each gland. Replacement was either by lymphocytes alone or by both fibrous tissue and lymphocytes. Patients with the mixed fibrolymphocytic type were somewhat older than patients with the lymphocytic type. Female patients showed greater replacement than male patients in each decade. With greater degrees of replacement the basal metabolic rates were lower and the incidence of auricular fibrillation was less. The incidence of exophthalmos increased progressively with increasing degrees of replacement. The incidence of postoperative myxedema rose progressively with the degree of parenchymal replacement. In cases showing 40 to 50 per cent replacement the incidence of postoperative myxedema approached 70 per cent.

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NONENCAPSULATED SCLEROSING TUMORS OF THE THYROID*

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AS early as 1927, Allen Graham (1) recognized the existence of a circumscribed but not encapsulated tumor of the thyroid which he designated as an adenocarcinoma not originating in an adenoma. Though he did not publish the cases in detail, his diagnosis of such lesions in the pathologic files of the Cleveland Clinic continues consistently from that date. Early in his studies of these tumors he recognized their particular nature and relative innocence. In pathologic reports he would often note that though malignancy was indicated by local invasion of stroma and adjoining thyroid there was no invasion of blood vessels and the prognosis was good. He twice observed these small tumors in thyroids removed at autopsy and found many of them incidentally in thyroids removed surgically for various reasons. In no case was the presence of the tumor suspected prior to operation or autopsy.

This report deals with 25 of these small, nonencapsulated tumors of the thyroid occurring between the years 1928 and 1941 at the Cleveland Clinic, emphasizes their particular nature and suggests that, until more is known of their natural history, they should not be regarded as true carcinoma.

REVIEW OF THE LITERATURE

The first and most specific description is that by Allen Graham. He described 6 cases of a small nonencapsulated tumor, less than 1 centimeter in diameter, arising in the nonadenomatous portion of nodular goiters. These he grouped under the name adenocarcinoma not originating in adenoma. Two of the 6 cases showed no recurrence in eleven and twelve years, the other 4 were too recent for evaluation. A seventh and larger tumor, which recurred after incomplete removal, was described in the group but Graham has recently stated (2) that it should not have been included. The tumors were described as glandular or glandulo-papilliferous and locally invasive. Malignancy was considered of very low order and the tumors were regarded as belonging to the most favorable group of carcinoma of the thyroid.

Goetsch (3) discussed 9 cases of small tumors, usually with dense fibrosis

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and even partial calcification, which he designated as carcinomatous lesions occurring in diffuse hyperplastic goiter. It was his belief that the lesions arose in adenomata and that the concept of truly primary carcinoma arising in a hyperplastic thyroid was not based on firm premises.

Pemberton and Black (4) reported 22 cases of carcinoma of the thyroid gland found incidentally in patients with exophthalmic goiter. Fifteen were classed as carcinoma not in adenoma and, of these, 14 were small (6.7 ± 1 mm.) low grade papillary tumors, at times multicentric, and in all but 2 instances confined to the thyroid. A follow-up study was reported on these papillary adenocarcinomas and 7 revealed no recurrence in one to six years after subtotal thyroidectomy. It was the opinion of the authors that the tumors could well have developed before the exophthalmic goiter without causing symptoms and also that the finding of a carcinoma in the thyroid of a patient who had received goitrogenic drugs did not imply relationship.

In so far as can be determined from a review of the literature, this group of small, usually papillary neoplasms has been separated as an entity only by Graham (1), who likewise is the first to recognize them as innocuous (5). The tumor in its usual form is so typical that there can be no question that the 9 instances of minute carcinomatous lesions considered by Goetsch, and the majority of the small papillary tumors described by Pemberton and Black belong to this same group.

CLINICAL FEATURES

In all instances the finding of the tumor was incidental to removal of the thyroid gland for other reasons. Five of the patients were men and 20

TABLE 1. NONENCAPSULATED SCLEROSING TUMOR
25 cases

Nodular goiter with hyperthyroidism	10
Diffuse goiter with hyperthyroidism	9
Nodular goiter without hyperthyroidism	2
Diffuse goiter, colloid	3
No disease	1

were women. The average age of the men was 47 years and that of the women 41 years. Nineteen of the 23 surgically removed thyroids were hyperplastic and the patients were operated upon for hyperthyroidism. These included 10 nodular and 9 diffuse goiters (Table 1). The operations consisted of thyroidectomy and in a few instances, lobectomy. Postoperative radiation therapy of 1,200 to 2,400 r. was given in a few of the earlier

cases (Table 2). Follow-up studies were possible on a majority of the patients and all of these were living without recurrence of the tumor five years or longer following operation (Table 2).

TABLE 2. NONENCAPSULATED SCLEROSING TUMOR OF THE THYROID
23 clinical cases

Treatment	Total no. of patients	No recurrence (5–10 yrs.)	No recurrence (over 10 yrs.)	Lost, or inadequate follow-up
Thyroidectomy only	20	7	7	6
Lobectomy only	0	0	0	0
Thyroidectomy and radiation	2	0	2	0
Lobectomy and radiation	1	1	0	0

PATHOLOGIC FEATURES

As indicated above, the majority of goiters were of the hyperplastic type. However, in 2 instances the neoplasms were found in nodular goiters without hyperplasia and in 3 instances in diffuse colloid goiters (Table 1).

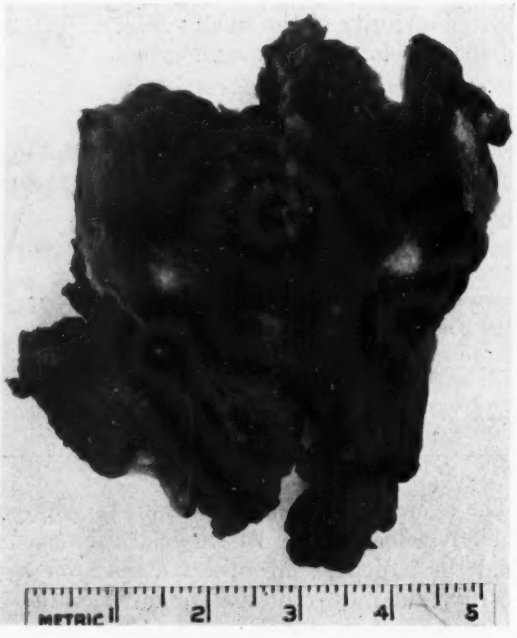

FIG. 1. White, scar-like tumor within thyroid (Case 4).

In one of the thyroids in which the lesion was found incidentally at autopsy there was no other gross or microscopic abnormality. The weight of the glands varied from 12 to 137 Gm. The 23 tumors found in the course of the examination of surgical material were encountered among 12,614 thyroids, an incidence of 1 in 522 or 0.2 per cent. The two lesions found at autopsy occurred in a series of 645 examinations of the thyroid, an incidence of 1 in 322.

FIG. 2. Irregular, white, nonencapsulated tumor of thyroid adjoining the capsule (Case 7).

Gross appearance: The capsular surfaces of the thyroid lobe were smooth, of usual color or, when the lesion adjoined the capsule, with an indefinite small pale patch. Sections revealed a small, firm or hard, white, yellowish or gray, circumscribed but not encapsulated lesion, often much like a scar, in the thyroid tissue (Fig. 1) but in many instances approximating the capsule of the gland (Fig. 2). In no case, however, did the tumor extend into tissues adjoining the thyroid. The lesion varied from 2 to 20 mm. in diameter with an average of 8.6 mm. The general contour varied from irregularly rounded to somewhat stellate. The tumor was often of gristle-like consistency and in 2 instances was grossly partially calcified. The cut surface was flat, smooth or finely granular and usually yielded no noticeable tissue when scraped. In this series the lesions were recorded as solitary (Table 3).

TABLE 3. NONENCAPSULATED SCLEROSING TUMOR OF THE THYROID
25 cases
GROSS APPEARANCE

Size	Under 20 mm.	
Shape	Ill-defined, nonencapsulated, circumscribed	
Color	White, gray, grayish-yellow	
Consistence	Firm, gristle-like, hard	
Location	Adjoining capsule	18
	Within thyroid lobe	7

Microscopic examination: In all but 1 of the cases the tumor was papillary, with acinar elements of variable size. Usually there was an abundant connective tissue stroma, at times of quite dense character. Calcification occurred rarely and was of patchy or psammomatous type. The epithelial cells were columnar or cuboidal and in a majority of instances, of small or medium size, 5 to 12 microns (Fig. 3a). They presented a pale-staining neutrophilic cytoplasm of moderate amount, thus resembling the usual cell type found in the papillary carcinomas which metastasize to the lateral cervical region. At times the epithelium was of tall character (Fig. 3b), up to 25 microns in height, and with a fairly abundant pink-staining cell cytoplasm. Occasionally there was a dense infiltration of lymphocytes and plasma cells in the stroma (Fig. 3c) of the tumors of tall-cell type. The epithelial cells usually were well differentiated. Lumina varied from small to moderate size, often containing no colloid. Mitoses were seldom found. Giant nuclear forms were present in an oxyphilic zone of one tumor (Table 4).

TABLE 4. NONENCAPSULATED SCLEROSING TUMOR OF THE THYROID
25 cases
MICROSCOPIC APPEARANCE

Predominant configuration:	Papillary		24
	Slight	5	
	Moderate or marked	19	
	Nonpapillary		1
Cell type:	Papillary, adeno		12
	Papillary, tall cell		10
	Papillary, tall cell with lymphocytes		2
	Nonpapillary, adeno		1
Apparent invasiveness:	Capsule alone		5
	Thyroid alone		13
	Thyroid and capsule		6
	None		1

The thyroid tissue adjoining the tumor was invaded slightly in 18 instances and moderately once. Though definite encapsulation was never present, the thyroid parenchyma was generally well demarcated from the major tumor mass. The most characteristic low power microscopic con-

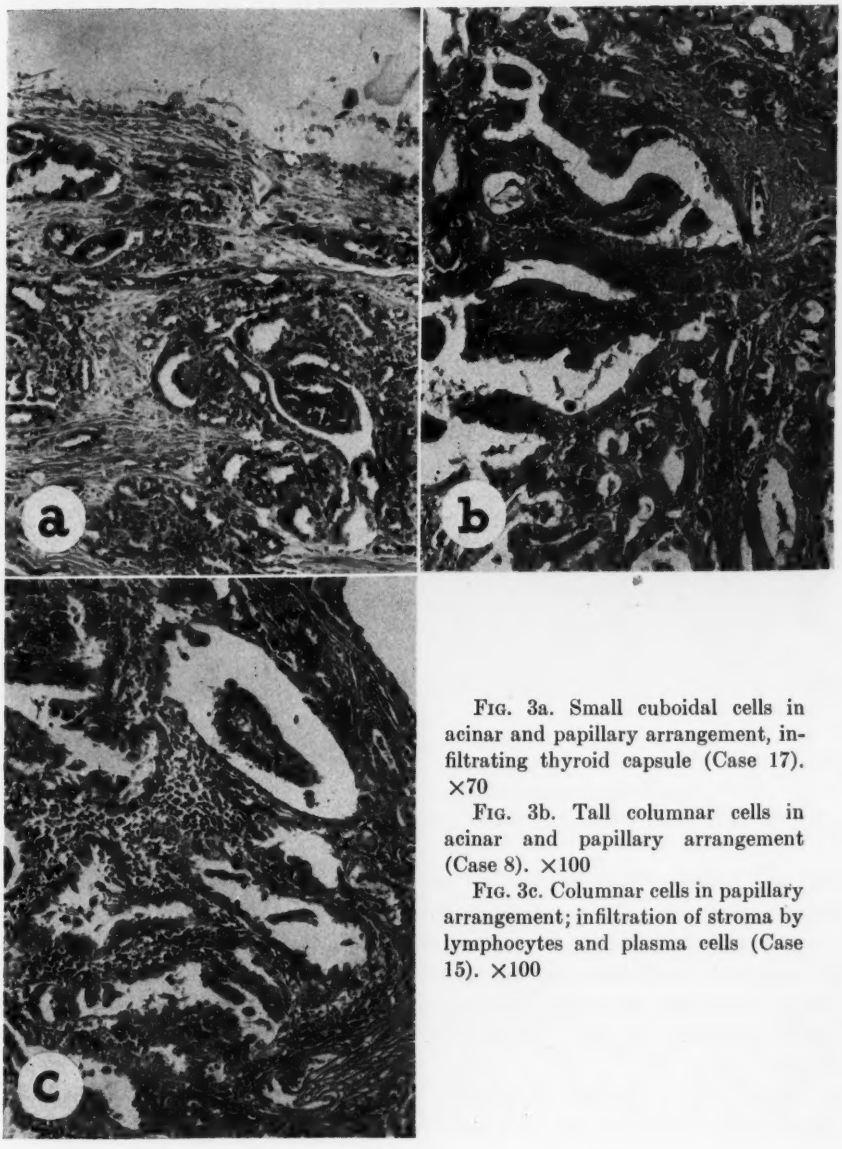

FIG. 3a. Small cuboidal cells in acinar and papillary arrangement, infiltrating thyroid capsule (Case 17). ×70

FIG. 3b. Tall columnar cells in acinar and papillary arrangement (Case 8). ×100

FIG. 3c. Columnar cells in papillary arrangement; infiltration of stroma by lymphocytes and plasma cells (Case 15). ×100

figuration was that of an irregularly rounded papillary tumor mass with abundant fibrous stroma marginated by thyroid lobules arranged so as to produce a scalloped appearance (Fig. 4a) and separated by irradiating bands of stroma continuous with that of the neoplasm. The lobules often appeared narrowed as they adjoined the neoplasm, producing a puckered appearance (Fig. 4b). In no instance was a compression zone apparent in the adjoining thyroid tissue. Infiltration of the thyroid occurred as single or small foci of epithelial elements intermingled with or replacing the

FIG. 4a. Nonencapsulated tumor showing scalloping of margin and sclerosis (Case 12). ×15
FIG. 4b. Nonencapsulated tumor showing sclerosis, papillary foci and puckered appearance (Case 16). ×15

thyroid follicles (Fig. 5a) and as extensions into the stroma of the gland, never exceeding a few millimeters.

The capsule adjoined the tumor in about two-thirds of the cases (Table 3) and in 11 instances was microscopically infiltrated, although there was never extension into the tissues adjoining the thyroid (Fig. 5b). Tumor cells infiltrated about a capsular blood vessel and into a nerve in 2 instances (Fig. 5c).

Differential pathologic diagnosis. Since these tumors are nonencapsulated and usually show invasion, even though slight, of the adjoining

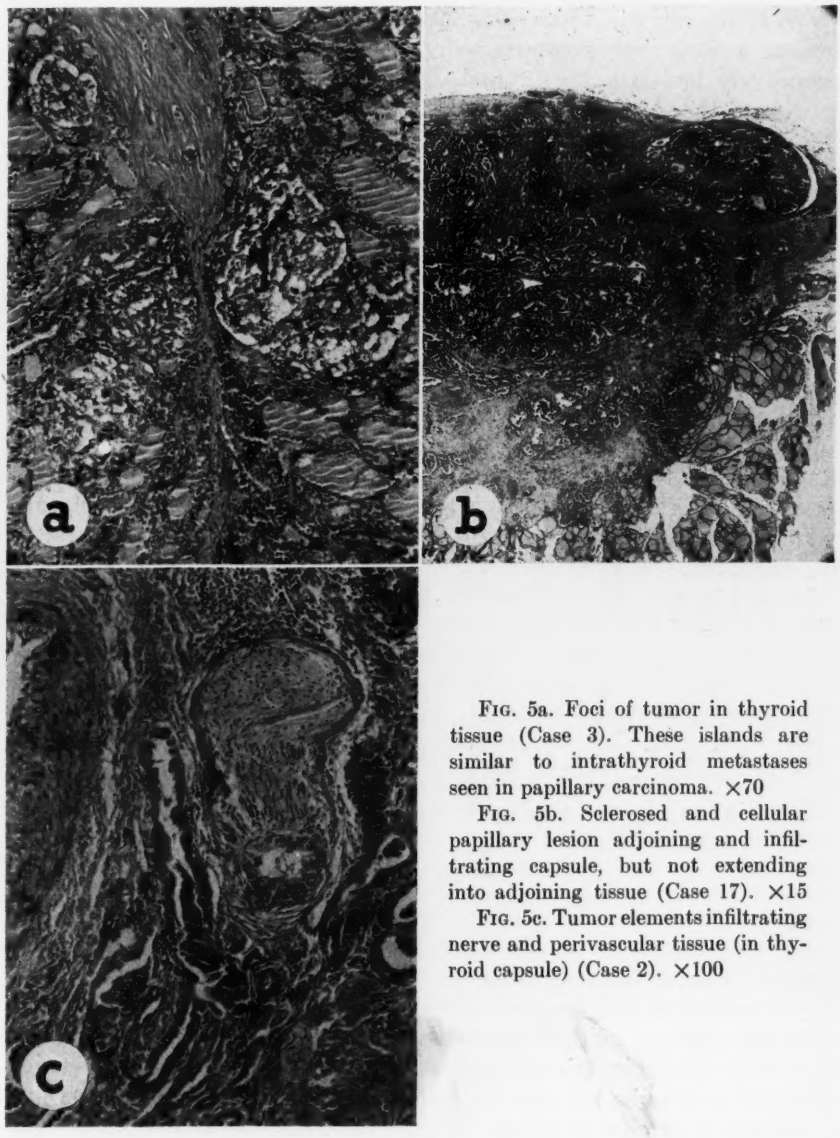

FIG. 5a. Foci of tumor in thyroid tissue (Case 3). These islands are similar to intrathyroid metastases seen in papillary carcinoma. ×70

FIG. 5b. Sclerosed and cellular papillary lesion adjoining and infiltrating capsule, but not extending into adjoining tissue (Case 17). ×15

FIG. 5c. Tumor elements infiltrating nerve and perivascular tissue (in thyroid capsule) (Case 2). ×100

thyroid or thyroid capsule, there has been a tendency to classify them as adenocarcinoma, usually of papillary type. Differentiation from those tumors which possess metastatic potentialities is of considerable importance. It will be noted that the nonencapsulated sclerosing tumors are

small, in this series not exceeding 20 mm. Though not encapsulated, they present a somewhat circumscribed appearance and do not at any time extensively infiltrate the thyroid. Though adjoining or infiltrating the capsule of the thyroid gland, they never invade the surrounding tissues. The microscopic configuration of the individual cells and of the luminated structures of the tumor is of no aid in differentiation since it approximates in all ways the variants found in true papillary carcinoma of the thyroid. In many lesions the low powered microscope view is of greatest importance as it reveals the scalloped margin of the adjoining thyroid lobules or the puckered appearance, both of which are distinctive as compared with papillary carcinoma. In those instances in which these features are not definite, the degree of infiltration never approaches that found in the usual papillary carcinoma nor is there a compression effect to give evidence of expanding tumor. Though there may be extension to blood vessel adventitia on a rare occasion, invasion of the vessel lumen is never found. Rarely, slight to moderate anaplasia may occur without increase in aggressiveness. Sclerosis is usually a pronounced feature in these neoplasms.

In summary, the major features in recognition of the tumors are: 1) the small size—in this series under 20 mm., 2) indolence of growth as evidenced by the slight infiltrative character and sclerosis, 3) the absence of blood vessel invasion, and 4) the absence of extension to tissues adjoining the thyroid despite involvement of the thyroid capsule.

It is quite probable that there is a small intermediate group in which differentiation is difficult between the apparently innocuous nonencapsulated sclerosing tumor and the papillary carcinoma with potentialities of both recurrence and metastasis. However, utilizing the points of differentiation above, these instances have been rare.

NOMENCLATURE

Since these tumors have been considered as an entity rarely, there is little preference as to name. Allen Graham designated them adenocarcinoma not originating in adenoma; Goetsch, as incipient carcinomatous lesions occurring in hyperplastic thyroids. Though both these names are probably justified, neither adequately describes the structure of the tumor as well as the clinical significance. Since the architecture and the benign behavior seem to be the only things that are positively known concerning this group of tumors, it is believed that the term used to designate them should indicate both of these qualities. As the term adenocarcinoma of the thyroid gland implies a poor prognosis, a feature not apparent in these tumors, the name adenocarcinoma not originating in adenoma seems misleading and would certainly cause confusion to those not familiar with

the neoplasm. On the other hand, since they may occur in thyroids which are not the seat of hyperplasia, as evidenced by several of the cases in this series, the term carcinoma in hyperplastic goiter inaccurately describes them. Because of these considerations, it has been deemed advisable to select a term which will omit reference to the tumors as carcinoma and yet will give some indication of their appearance. Since the features of nonencapsulation, of sclerosis and of tumor mass are prominent aspects of the lesion as viewed in the thyroid lobe, these descriptive terms have been combined to form the name proposed in this report, *nonencapsulated sclerosing tumor*. Though it is realized that an epithelial tumor of this type should be designated as adenoma or carcinoma, in view of present knowledge neither term can be accurately applied; the tumor apparently invades locally yet it does not appear to metastasize. Granted that epithelial tumors showing local evidence of invasion may be authentically designated as carcinoma, this term was avoided in order to prevent confusion in prognostic interpretations of this group of thyroid tumors, the exact nature of which is not yet known.

DISCUSSION

The genesis of the lesion has not been established. Goetsch strongly suspected that it originated in an adenoma and in several of his reported cases noted a central adenomatoid configuration. Moore suggested that such lesions may be caused by the use of antithyroid drugs (6). Pemberton and Black regarded them as papillary carcinomas developing from extra-adenomatous tissue and without connection with either exophthalmic goiter or with the use of goitrogenic drugs.

In no instance in this series was there any evidence of origin in an adenoma. The absence of a capsule about the lesions suggests two alternatives: 1) it is an embryologic rest incidentally found in the thyroids removed and 2) it is a slowly growing neoplasm, because of its indolence showing only slight invasion of adjoining tissue and remaining localized because of lack of aggressiveness. Proof of either of these contentions is not entirely possible.

The average age of the surgical patients who had this tumor is approximately the same as that of all patients operated on for goiter. This consideration and the possibility of the same relative incidence in surgical and autopsy material suggest that the tumor is, or is derived from, a congenital rest that occurs in a certain proportion of the entire population regardless of age or the presence of other disease of the thyroid gland. Thus it is possible that papillary tumors of the thyroid and especially those which occur in children and young adults and which metastasize to the

lateral cervical region originate from, or are a slightly more malignant form, of this tumor. Such a theory of origin for papillary tumors would explain not only the nonencapsulated sclerosing tumors but also the multicentric and bilateral involvement of the thyroid by papillary tumors, the youth of the patients affected by papillary carcinomas and the good prognosis. The nonencapsulated sclerosing tumor could be considered to be of the lowest degree of malignancy and yet to possess potentialities of developing into frank papillary carcinoma or of remaining harmless throughout life, undetected unless the thyroid is removed for some other cause. The relation is supported by the fact that the lesion closely simulates papillary carcinoma.

It is important that the innocence of the nonencapsulated sclerosing tumor of the thyroid be recognized and appreciated. Since its presence is never suspected clinically and it is rarely recognized at the time of operation, the surgeon may become unduly alarmed when the pathologist reports the presence of carcinoma. This may lead to reoperation, radical dissection of the neck or extensive irradiation, all of which are unnecessary and undesirable. If the true nature of the tumor is recognized its presence can be disregarded.

The incidence of this tumor in any institution will depend largely on how carefully the pathologist seeks for it. Every little area of scarring in the thyroid must be sought out and sectioned if these tumors are to be found.

The fact that the lesions have been reported with more frequency in hyperplastic goiters has led to their association with Graves' disease, or with the use of antithyroid drugs. Though the tumors may appear to occur with a little higher frequency in diffuse hyperplastic goiters than in the adenomatous type, this may be explained by the greater conspicuousness of the lesion in a diffuse hyperplastic gland. In multinodular goiters, scarring may be so extensive that the tumor is not recognized. During the same period of time that the 23 nonencapsulated sclerosing tumors were found in surgically removed glands, approximately one-fourth of the thyroidectomies were performed for simple goiter and three-fourths for hyperthyroidism. Four of the tumors found in surgical specimens were from patients who had no hyperthyroidism. Though this small series is inadequate for statistical evaluation it suggests that the incidence of the tumors may parallel that of the operations for simple goiter and for hyperthyroidism. In no instance in this series had thiouracil derivatives been used in the therapeutic management of a patient.

Regardless of the nature or the origin of the nonencapsulated sclerosing tumor of the thyroid, it is apparent that it is of the lowest order of malignancy and if discovered in a thyroid specimen need cause no alarm or alteration of the treatment.

Case Reports

Case 1. A man 26 years of age had had symptoms of hyperthyroidism for two months, about a year previously. Just before entry he had been taking desiccated thyroid. There was slight enlargement of the thyroid gland and the basal metabolic rate, fifteen days after he had stopped taking the thyroid, was plus 57 per cent. A thyroidectomy was performed; the specimen weighed 30 Gm. and showed diffuse follicular hyperplasia. A small papillary and moderately sclerotic, nonencapsulated tumor was present in one lobe. The patient was living and without recurrence of tumor eight years following operation.

Case 2. A woman 56 years of age had had a loss of weight during the preceding year. The thyroid was slightly enlarged. A basal metabolic rate was plus 47 per cent. A thyroidectomy was carried out, removing 15 Gm. of tissue showing diffuse hyperplastic goiter. In one lobe adjoining and including the capsule there was a nonencapsulated tumor, white with partly granular surface approximately 1 cm. in diameter, partly sclerotic and of adenopapilliferous structure. The patient was living without recurrence seventeen years after operation.

Case 3. A woman 28 years of age had had soreness in the region of the thyroid and nervousness for one week. The thyroid was markedly enlarged, very hard or firm, especially the right lobe. The basal metabolic rate was plus 59 per cent. A thyroidectomy was performed and the removed tissue weighed 85 Gm. The specimen showed follicular hyperplasia, several small nodules, and a nonencapsulated, partly sclerotic papillary tumor 15 mm. in diameter, immediately beneath the capsule in the middle outer anterior border of the right lobe. Postoperatively, 2,400 r. units of radiation therapy was given. The patient was living and without recurrence of neoplasm fourteen years following operation.

Case 4. A woman 36 years of age had had a goiter for five years and was without symptoms except for increase in size of the thyroid. There was nodular enlargement of both thyroid lobes. The basal metabolic rate was plus 32 per cent. A thyroidectomy was performed and produced 170 Gm. of tissue showing nodular goiter with slight hyperplasia. In the center of nonadenomatous thyroid tissue there was a small localized, nonencapsulated, firm, white nodule, cellular and moderately sclerotic, 4.0 mm. in diameter, with an adenopapilliferous architecture (Fig. 1). The patient was living without recurrence fifteen years following operation.

Case 5. A woman 54 years of age had had symptoms of hyperthyroidism for two months. The thyroid was enlarged and soft with a bruit. A thyroidectomy was performed. Pathologic examination revealed a diffuse hyperplastic goiter weighing 42 Gm. A moderately sclerotic, glandulo-papilliferous, nonencapsulated tumor, 7.0 mm. in diameter, was located in the middle of a lobe. The patient was living and well fifteen years following operation.

Case 6. A woman 58 years of age had had symptoms of hyperthyroidism for five years. The thyroid was approximately twice the normal size, smooth and firm. The basal metabolic rate was plus 47 per cent. At thyroidectomy 57 Gm. of tissue was removed, which showed nodular hyperplastic goiter. In the lower pole there was a moderately sclerotic and papillary nonencapsulated tumor, 1.0 cm. in diameter, with one irregular calcified area. There was no recurrence of neoplasm one year following operation; follow-up beyond this period was not possible.

Case 7. A woman 40 years of age, who had a goiter for approximately twenty-five years, developed rapid enlargement of the thyroid and symptoms of hyperthyroidism during the year before entry. There was nodular enlargement of the thyroid and mod-

erate exophthalmos. A thyroidectomy was performed. The specimen weighed 80 Gm. and revealed nodular hyperplastic goiter. In the mid-portion on the anterior capsule of the left lobe there was a firm, grayish-tan nodule, 15 mm. in diameter, nonencapsulated and of adenopapilliferous type (Fig. 2). A course of radiation therapy totalling 1,200 r. was given postoperatively. Fifteen years after operation the patient re-entered because of a recurrence of a nodule in the thyroid site. This was excised from the pyramidal lobe and was an adenoma, slightly hyperplastic, with no evidence of malignancy.

Case 8. A man 56 years of age had had symptoms of exophthalmic goiter for three months. The thyroid was symmetrically enlarged, about one and one-half times normal and was firm. The basal metabolic rate was plus 45 per cent. A thyroidectomy was performed and 24 Gm. of tissue was removed, which revealed diffuse hyperplastic goiter. In the upper pole of the left lobe there was a firm, yellowish, nonencapsulated nodule 4.0 mm. in diameter, sclerotic and papillary (Fig. 3b). The patient was living and without recurrence of tumor fifteen years following operation.

Case 9. A man 52 years of age complained of palpitation and weight loss during two months. The thyroid was not palpably enlarged. The basal metabolic rate was plus 15 per cent. A thyroidectomy was performed. The specimen weighed 50 Gm. and pathologic examination revealed diffuse hyperplastic goiter. Immediately beneath the capsule of the anterior surface near the lower pole there was a firm, whitish, nonencapsulated area 2.0 mm. in diameter, sclerotic and adenopapilliferous. There was no recurrence of tumor nineteen years following operation.

Case 10. A woman 57 years of age, had had symptoms of hyperthyroidism for one year. The thyroid was one and one-half times normal size, with two nodules in the midline. Thyroidectomy was carried out revealing nodular hyperplastic goiter. The specimen weighed 28 Gm. In the upper pole of the left lobe there was a small, nonencapsulated, firm, white mass, 4 mm. in diameter, with calcified plaque. Histologically it was sclerotic and papillary. There was no recurrence of tumor eighteen years later.

Case 11. A woman 47 years of age, had had symptoms due to progressive enlargement of the thyroid for six years. There was nodular enlargement of the thyroid gland to about four times normal size, but no thrill or bruit. The pulse rate was 104. A right lobectomy was performed and 100 Gm. of tissue was removed, which showed nodular goiter. Near the upper pole, a few millimeters below the anterior capsule, there was a firm, white, circumscribed nodule 6.0 mm. in diameter, of sclerotic and glandular architecture. A left lobectomy was performed two days later and revealed nodular goiter. Follow-up was not possible.

Case 12. A woman 32 years of age had had a goiter since adolescence and for a period of one year before entry had noticed nervousness, tiredness, shortness of breath and enlargement of the thyroid. The gland was enlarged to five times normal size. The basal metabolic rate was plus 49 per cent. A thyroidectomy was performed, involving the removal of 85 Gm. of tissue which revealed nodular goiter with hyperplasia. In the right lobe, situated about 1.0 cm. beneath the capsule of the lower pole there was a small white, nonencapsulated area, 7.0 mm. in diameter, firm, fibrous and with a sclerotic and irregularly glandular structure (Fig. 4a). To a letter of inquiry eight years after operation the patient replied that she had a "fullness" in her throat and believed the goiter was growing back. No more definite follow-up was possible.

Case 13. A woman 34 years of age had had symptoms of hyperthyroidism for one year. There was diffuse enlargement of the thyroid to twice normal size. A right lobectomy was performed, producing 12 Gm. of tissue from a diffuse goiter with slight hyperplasia. A small piece of tissue, attached to the right side of the trachea, was removed

separately and was formed by firm, gray tissue 1.0 cm. in diameter, of irregular contour and of moderately sclerotic and papilliferous architecture. Histologically, the neoplasm was surrounded by partially infiltrated thyroid gland. Postoperatively, radiation therapy was given, consisting of 1,950 r. units. The patient was living without recurrence five years following operation.

Case 14. A 55-year-old woman had had a lump in her neck for thirty-five years and during the ten weeks before entry had had symptoms of hyperthyroidism. The thyroid was hard, nodular, movable and four times the usual size. The basal metabolic rate was plus 47 per cent. A thyroidectomy was carried out, and 80 Gm. of tissue removed which showed nodular goiter with hyperplastic adenomata. In the superior pole of the left lobe there was a small whitish area in the capsule and adjoining internodular thyroid, moderately sclerotic and of adenopapilliferous architecture. No follow-up information could be obtained.

Case 15. A woman 43 years of age had known she had a goiter for two years, and had been aware of nervousness and excitability. The thyroid was three times average size and symmetrical. A basal metabolic rate was 43 per cent. A left lobectomy was performed, involving removal of 30 Gm. of tissue showing diffuse goiter, with slight hyperplasia. In the center of the lobe there was a fibrous scar, about 7.0 mm. in diameter, nonencapsulated, hard and extending inward from the anterior capsule. Histologically, there was dense sclerosis and papillary configuration. A right lobectomy was performed the following day revealing diffuse goiter, with slight hyperplasia. There was no recurrence of tumor eight years following operation.

Case 16. A woman 35 years of age experienced nervousness for two months and noted a great deal of weight loss. The thyroid was slightly enlarged. A basal metabolic rate was plus 65 per cent. A thyroidectomy was performed and 48 Gm. of tissue was removed, which showed diffuse goiter with slight hyperplasia. In one area there was a small fibrous, scarred area, with dense sclerosis and papillary configuration (Fig. 4b). The patient died six years later of tuberculosis of the kidney. There was no recurrence of neoplasm.

Case 17. A woman 23 years of age had had an enlargement of her neck for ten years. There was a nodule in the right lobe of the thyroid which was movable and nontender. The basal metabolic rates were minus 23 per cent and minus 32 per cent on two occasions. A thyroidectomy was carried out, removing 70 Gm. of tissue showing colloid goiter with adenoma and without evidence of hyperplasia. In the periphery of the left lobe, immediately beneath the capsule there was a nonencapsulated tumor 1.5 cm. in diameter which was fibrotic and presented a papillary architecture (Figs. 3a and 5b). There was no recurrence of neoplasm five years following operation.

Case 18. A white man 55 years of age had had symptoms of hyperthyroidism for six months. The thyroid was slightly enlarged. A basal metabolic rate was plus 15 per cent. A right lobectomy was performed and the specimen consisted of 18 Gm. of tissue, formed by nodular goiter. Beneath the capsule near the upper pole there was a nonencapsulated, firm white nodule, 7.0 mm. in diameter, showing slight sclerosis and a papillary configuration. A left lobectomy was performed three weeks later and revealed nodular goiter. There was no recurrence of tumor twelve years following operation.

Case 19. A woman 48 years of age had experienced gradual enlargement of the thyroid over a period of six years. The thyroid presented a small nodule in the isthmus, about 1.0 in diameter. There were no signs of hyperthyroidism. A thyroidectomy was performed, removing 30 Gm. of tissue showing nodular goiter. Along the posterolateral border of the right lobe and 1.5 cm. from the upper pole there was a nonencapsulated,

firm nodule immediately beneath the capsule, 1.0 cm. in diameter, with a partly granular cut surface, and histologically of adenopapilliferous configuration. The patient was alive nine years later, with carcinoma of the stomach, but without any evidence of recurrence of the thyroid tumor.

Case 20. A woman 57 years of age entered because of weakness, dyspnea, and sense of fullness and pounding in the neck. There was nodular enlargement of the thyroid. The basal metabolic rate was minus 2 per cent. A left lobectomy produced 35 Gm. of thyroid tissue typically nodular goiter and without evidence of hyperplasia. A right lobectomy the following day produced 40 Gm. of tissue, characteristic of nodular goiter and without hyperplasia. In the upper pole of the right lobe there was a localized, nonencapsulated, hard white area, 1.5 cm. in diameter, embedded in the thyroid tissue, with considerable sclerosis and a papillary configuration. No follow-up information could be obtained.

Case 21. A 25-year-old woman had had symptoms of hyperthyroidism for two years. The thyroid was diffusely enlarged and the eyes were prominent. The basal metabolic rate was plus 37 per cent. A thyroidectomy was performed and 35 Gm. of tissue removed which revealed a diffuse hyperplastic goiter. Beneath the capsule of the right lobe on the lateral border there was a well localized, nonencapsulated, firm translucent nodule, 8.0 mm. in greatest diameter which, on section, was slightly sclerotic and quite papillary. The patient was living without recurrence of tumor eight years after operation.

Case 22. A woman, 32 years of age, who had had a "large neck" since childhood, had noticed symptoms typical of hyperthyroidism for one year. The thyroid was enlarged bilaterally and seemed lobulated. The basal metabolic rate was plus 19 per cent. A thyroidectomy was performed, the specimen consisting of 42 Gm. of tissue, formed by a diffuse goiter, colloid type. On the posterior border of the left lobe there was a well localized, nonencapsulated, hard white tumor, 20 mm. in greatest diameter, involving the capsule and extending into the lobe, showing moderate sclerosis and a papillary configuration. A follow-up study could not be obtained.

Case 23. A woman, 54 years of age, had had an enlargement of the neck twenty years previously, and the year before entry began to have symptoms typical of hyperthyroidism. There was a nodular enlargement of the thyroid to three times the normal size, and the eyes were somewhat prominent. The basal metabolic rate was plus 37 per cent. A thyroidectomy was performed. The specimen weighed 137 Gm. and showed nodular goiter with hyperplasia. On the anterior surface of the right lobe near the lower pole there was a grayish-white area with ill-defined borders in the capsule, measuring 7.0 mm., papillary and moderately sclerotic. No follow-up information could be obtained.

Case 24. A woman, 20 years of age, was admitted in comatose state, following one week of severe illness characterized by frontal headache, loss of vision, nausea and vomiting. Death occurred on the day following admission and the major autopsy diagnosis was bilateral brain abscesses due to Streptococcus hemolyticus. Pathologic examination of the thyroid revealed a gland weighing 35 Gm. which was not remarkable except for a localized area in the left lobe, deep in the thyroid tissue consisting of a small round grayish-white, firm tumor, nonencapsulated and measuring 5.0 mm. in diameter. Histologically, the lesion was of papillary configuration with slight fibrosis.

Case 25. A man, 48 years of age, was examined at post mortem because of acute purulent peritonitis. Pathologic examination of the thyroid revealed a gland weighing 65 Gm. and showing some colloid distention of the follicles, but otherwise not remarkable except for the tracheal surface of the left lobe, near the lower pole, where there was a small, nonencapsulated grayish-white nodule, 8.0 mm. in diameter, within the thyroid but adherent to the capsule. Histologically, the lesion was of papillary configuration and with slight fibrosis.

SUMMARY

Twenty-five instances of a small tumor of the thyroid designated as nonencapsulated sclerosing tumor are presented. These were encountered incidentally in the examination of thyroids which in 23 instances were removed surgically for non-neoplastic goiter and in 2 were incidental findings at autopsy. In none of the clinical cases was there local recurrence or distant metastasis.

It is emphasized that these tumors are benign in their behavior. Theories as to genesis and the relation of these tumors to papillary thyroid carcinoma are considered.

The nonencapsulated sclerosing tumor should be recognized as a pathologic entity and patients with these tumors should not be subjected to radical operations or irradiation therapy.

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THE CURRENT TREATMENT OF HYPERTHYROIDISM*

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OF the recent developments in the field of thyroid research two have proved to be of far-reaching clinical significance: 1) the discovery that the function of the thyroid gland can be suppressed by goitrogenic drugs, notably thiouracil and related compounds, and 2) the demonstration of the value of radioiodine as a tool for study of thyroid function as well as a therapeutic agent in the treatment of some types of thyroid disease. By opening new approaches to thyroid investigation, these agents have been responsible for rapid and sound advances in our knowledge of the physiology of the thyroid gland, and both have contributed greatly to the effectiveness of the treatment of hyperthyroidism. However, it is evident that these advances have not provided the final solution to the problem of goiter, nor has effectiveness of treatment yet reached the stage whereby established methods of treatment can now be discarded. It seems obvious that the present status of surgical treatment of hyperthyroidism cannot be accurately appraised without reference to the role these newer agents are now playing in the over-all management of toxic goiter.

It is the purpose of this discussion to review our clinical experience with these agents at the Mayo Clinic, and to attempt to evaluate their use in the treatment of hyperthyroidism.

GOITROGENIC COMPOUNDS

Several investigators have employed thiouracil or propylthiouracil as total treatment in the hyperthyroidism of exophthalmic goiter. Although complete remission of hyperthyroidism in most cases can usually be induced in two to three months and apparently maintained as long as administration of the drug is continued, the incidence of recurrence of the disease after withdrawal of the drug is high. Thus, Williams (1) reported that two weeks to five months after administration of the drug was discontinued, 51 per cent of his patients had relapse of the disease. In a series of 17 cases reported by McCullagh and his associates (2), the disease recurred in 6 (35 per cent) in one week to fourteen months after the withdrawal of the drug.

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The high incidence of recurrence after treatment with goitrogens, the difficulty in keeping patients under prolonged observation and the occurrence of occasional untoward reactions, even from use of propylthiouracil (3) are the factors that have influenced us to confine the use of the drug as total treatment for hyperthyroidism to the occasional, unusual case in which no other form of treatment seems adequate or feasible. Furthermore the relatively small but significant incidence (4 to 6 per cent), of malignant lesions in nodular goiters definitely contraindicates the employment of any nonsurgical method as total treatment for hyperthyroidism associated with adenomatous goiter, except in rare instances.

As a measure of preparation for operation, propylthiouracil used concurrently with iodine has been valuable in reducing the operative mortality rate in those cases in which the patient presents a poor surgical risk because of the severity of the hyperthyroidism or because of the presence of complications. The question arises whether there are reliable criteria by which the safety of the operation on patients with toxic goiter prepared by medication with iodine can be accurately judged. In other words, can we separate the good risks from the bad? An analysis of the potential hazards associated with the surgical treatment of these patients reveals that they fall into two main groups: 1) the normal hazards inherent in any surgical procedure, such as those connected with operation on patients with nontoxic goiter—these are limited, for the most part, to technical errors—and 2) the additional hazards incident to hyperthyroidism. These additional hazards in turn may be divided into two subgroups: a) those associated with the development of an overwhelming postoperative hyperthyroid crisis, usually confined to cases of severe hyperthyroidism, and b) those related to the debility of the patient that results from the intensity of the disease, long-continued hyperthyroidism, advanced age or intercurrent disease.

On the basis of these potential hazards is it possible to gauge, with a reasonable degree of accuracy, the surgical risk for the patient with toxic goiter? As previously reported (4), an attempt to determine this point was made by correlating the mortality rate with the previously recorded ratings of the hazards in a large number of patients with toxic goiter prepared with strong iodine solution (Lugol's solution). The grading of the operative risk was made on the basis of 1, 2, 3 and 4. The normal risk of the patient with nontoxic goiter was graded 1, and the normal risk of the patient with toxic goiter was graded 1+. The estimation of any additional hazard was added to the normal rating. From this survey it was learned that although it is not possible to foretell which patient will not survive the operation, it is possible from the clinical estimate of the hazard to select a small group of patients whose ratings are adjudged moderately or greatly increased

and from whom 81 per cent of the mortality will be derived.

Since the time required for effective preparation with goitrogenic drugs, of the patient who has exophthalmic goiter, is two or three times greater than that needed for adequate preparation with strong iodine solution; since the goitrogenic drugs are potentially toxic; and since the operative risk in cases of mild or moderate severity in which the patient is prepared with strong iodine solution is no greater than it is in cases of goiter unassociated with hyperthyroidism, it is our practice at the clinic to confine the use of propylthiouracil to the cases of severe or complicated disease. The results of surgical treatment of patients with toxic goiter prepared as outlined have been excellent. Thus, at the clinic in a recent series of 611 consecutive cases of exophthalmic goiter in which thyroidectomy was performed there were no deaths. In a similar series of 496 patients who had adenomatous goiter associated with hyperthyroidism and who underwent operation during the same period, 2 patients died. Thus, the mortality rate for the combined groups, comprising all the cases of hyperthyroidism, was only 0.18 per cent (Table 1). Subtotal thyroidectomy was performed as a

TABLE 1. DATA ON 2,351 CONSECUTIVE CASES OF GOITER (TO JANUARY 1, 1949) IN WHICH THYROIDECTOMY WAS PERFORMED

Diagnosis	Cases	Hospital deaths		
		Number	Per cent	
Exophthalmic goiter	611	0	0	0.18
Adenomatous goiter with hyperthyroidism	496	2	0.4	
Adenomatous goiter without hyperthyroidism (including thyroiditis)	1,131	0	0	
Malignant disease	113	1	0.88	
Total	2,351	3	0.13	

single-stage procedure in all the cases of exophthalmic goiter and in all but 3 of the cases of adenomatous goiter. In all these 3 cases technical difficulties, rather than inadequate preparation of the patient, constituted the factor which influenced the decision to perform the thyroidectomy in stages.

Data regarding the number of patients to whom the goitrogenic drug was administered are not readily available, but it is estimated that the number did not exceed 5 per cent of the patients who underwent operation. Those patients who were prepared with propylthiouracil for operation were, as a group, patients who were severely hyperthyroid or who had complications which were being adversely affected by hyperthyroidism.

In such cases prolonged periods of preoperative preparation seemed justifiable and the complete control of hyperthyroidism before operation was undoubtedly an important factor in the absence of mortality in the group. In rare cases, toxic manifestations of the drug necessitated return to older methods of preparation.

Although the number of cases in which we have used propylthiouracil in the preparation of the patient with hyperthyroidism for operation is small, we are confident that it has made a very valuable contribution to preoperative treatment. Other measures of proved value introduced in recent years also have greatly contributed to reducing the mortality rate in all fields of surgery. Agents for controlling infections, such as the sulfonamide compounds and the antibiotics, are outstanding examples. Thus, in a group of 1,131 patients with nontoxic goiter who underwent thyroidectomy without any special preoperative preparation, there were no deaths.

RADIOIODINE

Radioiodine has proved to be an effective means of control in many cases of hyperthyroidism. Its use in this respect continues to be cautiously explored. No serious ill-effects following the use of radioiodine have yet been reported, but concern over the possibility that untoward consequences of radiation might appear long after administration of radioiodine is an important factor that limits its more extensive use in hyperthyroidism at present.

Because of the unknown character of the radiation hazard in the treatment of hyperthyroidism with radioiodine, at the clinic we have deliberately limited the use of radioiodine to older patients for whom thyroidectomy is regarded as unduly hazardous because of some serious complication such as heart disease, and to cases in which repeated recurrences have followed previous thyroidectomies. The patients selected have, therefore, included a group in which the hyperthyroidism had proved itself resistant to other forms of treatment. Insufficient time has elapsed for accurate evaluation of the results of treatment of hyperthyroidism with radioiodine. To date, however, the disease has been satisfactorily controlled by this means in more than 80 per cent of the patients selected. The incidence of recurrences and of myxedema following use of radioiodine has, as might be expected, been found to be higher than is the incidence following thyroidectomy, but in those patients in whom the disease has recurred, further doses of the isotope have controlled the disease. Much remains to be learned regarding the effective dose. Whether or not this can be gauged so accurately that recurrence or myxedema becomes less frequent, it seems clear at present that this substance will hold for some time an important

place in the treatment of many patients in whom the risk of thyroidectomy would be excessively high even after preoperative preparation with propylthiouracil.

Patients with recurrent exophthalmic goiter in whom the thyroid remnant has not appreciably enlarged seem to us to be peculiarly suitable for treatment with radioiodine, as further surgical intervention will result in the removal of only minute amounts of thyroid tissue, and as these patients are particularly prone to recurrence of hyperthyroidism after treatment with antithyroid drugs. Elderly patients with hyperthyroidism and serious heart disease are also good candidates for radioiodine therapy, as no immediate risk has resulted in such cases, and late effects of radiation are not feared in such cases as they are in the young patient.

Radioiodine, either as a research tool or as a therapeutic agent, is potentially dangerous and its use requires the same kind of precautions against the insidious and disastrous consequences of radiation for the patient, the physician and the technician as does the use of radium or roentgen rays. The clinical application of radioiodine involves a large number of intricate and often highly technical problems, so that for a considerable time to come the safe use of this agent will be possible only when supervised by well-integrated research teams which include physicists, radiologists, biochemists, internists and surgeons.

SUMMARY

Propylthiouracil is a valuable drug for controlling hyperthyroidism, but because of its potential toxic side effects and because of the high incidence of relapses after its withdrawal, employment of this agent as the method of choice in the total treatment of exophthalmic goiter has, in our opinion, not been established.

As a means of preparing the patient for operation, medication with iodine has proved adequate except for a small group of patients whose operative risk is high because of severity of hyperthyroidism or because of complications. For this group, treatment with propylthiouracil has been found to be very valuable as an additional measure of preparation. The surgical mortality rate for patients with toxic goiter prepared according to the foregoing method has been exceedingly low; namely, 0.18 per cent.

Treatment with radioiodine has proved to be an effective means of controlling the disease in many cases of hyperthyroidism. The permanence of the results cannot now be determined. Until greater accuracy in gauging the dose is established and until the potential radiation hazard in the treatment of hyperthyroidism with radioiodine is known, we believe that caution demands that employment of this drug be limited to those patients for whom thyroidectomy is regarded as unduly hazardous.

We feel that thyroidectomy after preparation with either strong iodine solution alone or strong iodine solution and propylthiouracil is at the present time the most conservative method of treatment for the majority of patients with exophthalmic goiter. The short time needed for this method of treatment, the speed of control of hyperthyroidism, the high incidence of permanence of control, and the low incidence of morbidity and mortality make surgical treatment a method which must still be considered in most cases of hyperthyroidism and which will be the treatment of choice in many instances.

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EDITORIAL

JOURNAL OF CLINICAL ENDOCRINOLOGY

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MORE LIGHT ON THE BIOCHEMISTRY OF DIABETES

MOST endocrinologists would concede without argument, perhaps, that the most fundamental problem in our field is the relation of hormones to the cytologic processes in target-tissue cells. There are several theoretical possibilities as to the mechanism of intracellular action of internal secretions. The given hormone could serve merely as an immediate catalyst or as a factor in an enzyme system without entering directly into either the anabolic, catabolic, or discharge processes. It could serve stoichiometrically as an intermediate agent or as a final component in any of the chemical reactions upon which all functions depend. By either of these processes, it could influence the degree of responsiveness of the cell to nervous impulses or to circulating metabolites. Likewise it could affect the functions by altering the permeability relationships in the cell membranes. In any case, much more and better evidence is needed as to where hormones go and what they do when they get there.

The Department of Biological Chemistry at the Harvard Medical School has started, under the direction of Professor A. Baird Hastings, an extensive program of studies on the regulation of protein, fat and carbohydrate metabolism, including the influences of hormones on these processes. Attention has recently been focussed on carbohydrate metabolism. Radioactive carbon-labeled substances can be injected into experimental animals or applied to the tissues directly and the rates of formation and of breakdown of specific organic compounds thus followed precisely rather than being deduced—with questionable reliability—from end products, as has mostly been our only recourse in the past.

Throughout the period since the discovery of insulin, repeated attempts have been made to determine what chemical processes are influenced by it and whether it acts primarily by slowing the formation of sugar or by the acceleration of its breakdown. In the Harvard studies, sugar labeled with radioactive carbon 14 was obtained by utilizing the synthesizing

abilities of either green leaves of bean plants which were allowed to assimilate labeled carbon dioxide, or of liver cells to which various intermediates were supplied. The course of this labeled sugar was then followed in the muscle (diaphragm) of rats that had been rendered diabetic or had been deprived of their pituitary or adrenal glands.

In vitro studies showed that the presence of insulin accelerated both glycogen synthesis and sugar combustion. As is well known, the combustion of sugar depends upon an intricate series of reactions for which energy is derived from phosphate bonds. As judged by the results of the glandular deficiencies, the hormones of the pituitary and the adrenals as well as insulin itself act on the first step in sugar utilization, namely, that in which phosphoric acid enters into the molecules. It appears that insulin accelerates this reaction, whereas the adrenal and pituitary hormones decrease it. The level of the blood sugar at any given time is a resultant of the joint action of these accelerating and retarding substances. Thus, the diabetic suffers an imbalance whereby the retarding substances gain an undue influence and, in compensation, the body cells obtain a greater portion of their energy from the burning of fat, with eventual acidosis (1).

Other studies were made using labeled acetic acid and pyruvic acid, which are evanescent intermediate products formed when fats and sugars are burned in the body. The utilization of both these substances was found to be decreased in muscle from diabetic subjects, but only in the case of pyruvic acid was utilization restored to normal by the addition of insulin. Thus, additional weight is thrown upon the acetic-acid factor in the genesis of diabetic acidosis. As the authors (2) suggest, if hormonal or other factors that will selectively promote the combustion of acetic acid can be found, not only will fundamental knowledge of the biochemical abnormalities of diabetes be promoted, but the possibility of better management of the disease may be anticipated.

R. G. HOSKINS

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Postgraduate Assembly in Endocrinology Including Diabetes

Sponsored by

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

and

THE AMERICAN DIABETES ASSOCIATION

MIAMI BEACH, FLORIDA — RONEY PLAZA HOTEL — APRIL 3–8, 1950

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders, from the United States and Canada.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

The Roney Plaza, one of Miami Beach's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON MARCH 3, 1950. Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Application for approval of this course has been made to the Veterans Administration. Veterans should make formal application to their local agencies on the appropriate form (1905e or 1950) as furnished by the V.A.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, before March 3, 1950. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida, and the hotel advised that you are attending this Postgraduate Assembly.

The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Doctors Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Those wishing to present papers, which will be limited to ten minutes, should send title and four copies of an abstract of not more than 200 words, to Edward A. Doisy, M.D., St. Louis University School of Medicine, 1402 South Grand Avenue, St. Louis, Missouri, not later than March 1, 1950. *It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* Names of non-members who are co-authors must be followed by the words "by invitation," and the principal degree of each author.

Nominations for the Ayerst, McKenna and Harrison Fellowship, the Schering Fellowship in Endocrinology, and the Squibb and Ciba Awards should be made on special application forms which may be obtained from the Secretary-Treasurer, Henry H. Turner, M.D., 1200 North Walker Street, Oklahoma City 3, Oklahoma, and filed with the Secretary not later than March 15, 1950.

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

The Ayerst, McKenna and Harrison Fellowship

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

The Schering Fellowship in Endocrinology

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

The E. R. Squibb and Sons Award

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

The Ciba Award

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for $1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

* * * * *

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950.*

The 1950 Meeting of the American Goiter Association

The next annual meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1950.

The program will include among its speakers leaders in all fields of thyroid disease. The Chairman of the Program Committee is Dr. Rulon W. Rawson, Memorial Hospital, 444 East 68th Street, New York 21, N. Y.

All physicians in good standing in their respective medical societies may attend the meeting by registering as attending members. All members or others planning to attend are urged to make their reservations immediately by addressing the Reservation Department of the Hotel Shamrock, Houston, Texas.

Award of the American Goiter Association

VAN METER PRIZE

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Houston, Texas, March 9, 10 and 11, 1950, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, and must be presented in English. A typewritten double spaced copy *in duplicate* should be sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East St. Vrain Street, Colorado Springs, Colorado, not later than *January 15, 1950.* The Committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.